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Interplay of YB-1 between Tubulin and mRNA

Directeurs de Thèse : **Lev P. OVCHINNIKOV** et **Patrick A. CURMI**

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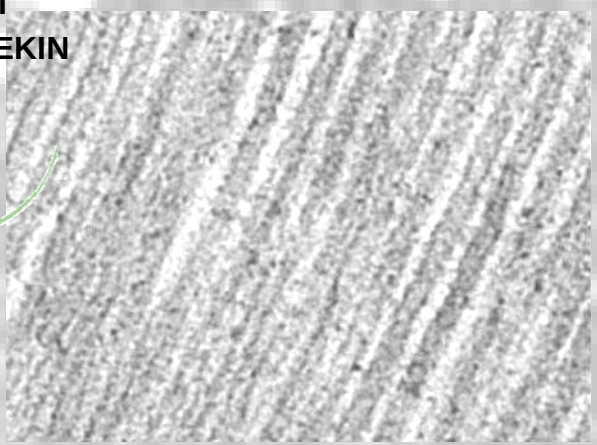


LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the original articles, which are mentioned in the text by their Roman numerals:

I) Chernov,K.G., Mechulam,A., Popova,N.V., Pastre,D., Nadezhdina,E.S., Skabkina,O.V., Shanina,N.A., Vasiliev,V.D., Tarrade,A., Melki,J., Joshi,V., Baconnais,S., Toma,F., Ovchinnikov,L.P., and Curmi,P.A. (2008). YB-1 promotes microtubule assembly in vitro through interaction with tubulin and microtubules. BMC. Biochem., 9, 23.

II) Skabkin,M.A., Kiselyova,O.I., Chernov,K.G., Sorokin,A.V., Dubrovin,E.V., Yaminsky,I.V., Vasiliev,V.D., and Ovchinnikov,L.P. (2004). Structural organization of mRNA complexes with major core mRNP protein YB-1. Nucleic Acids Res., 32, 5621-5635.

III) Chernov,K.G., Curmi,P.A., Hamon,L., Mechulam,A., Ovchinnikov,L.P., and Pastre,D. (2008). Atomic force microscopy reveals binding of mRNA to microtubules mediated by two major mRNP proteins YB-1 and PABP. FEBS Lett., 582, 2875-2881.

IV) Nekrasov,M.P., Ivshina,M.P., Chernov,K.G., Kovrigina,E.A., Evdokimova,V.M., Thomas,A.A., Hershey,J.W., and Ovchinnikov,L.P. (2003). The mRNA-binding protein YB-1 (p50) prevents association of the eukaryotic initiation factor eIF4G with mRNA and inhibits protein synthesis at the initiation stage. J Biol Chem, 278, 13936-13943.

V) Mechulam,A., Chernov,K.G., Mucher,E., Hamon,L., Curmi,P.A., and Pastre,D. (2009). Polyamine sharing between tubulin dimers favours microtubule nucleation and elongation via facilitated diffusion. *PLoS. Comput. Biol.,* **5,** e1000255.

ABBREVIATIONS

AAA ATPases associated with various cellular functions
AFM Atomic force microscopy
ATP adenosine triphosphate
CSD “cold-shock” domain
CSP “cold-shock” protein
CTP cytidine triphosphate
Dbp DNA-binding protein
DNA deoxyribonucleic acid
DRB D-ribofuranosyl-benzimidazole
EDC 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA ethylenediaminetetraacetic acid
eEF eukaryotic elongation factor
eIF eukaryotic initiation factor
EGTA ethyleneglycol-bis(β-aminoethyl ether)-N,N,N’,N’tetraacetic acid
hnRNP heterogeneous nuclear ribonucleoprotein
GTP guanosine triphosphate
IPTG isopropyl-β-D-thiogalactopyranoside
kb kilobase
K_D dissociation constant
kDa kilodalton
MAP microtubule associated protein
MT microtubule
NHS N-hydroxysulfosuccinimide hydrochloride
NSEP nuclease-sensitive element binding protein
nt nucleotide
NTP nucleoside triphosphate
ORF open reading frame
PABP poly(A) binding protein
PAGE polyacrylamide gel electrophoresis
RNA ribonucleic acid
RNase ribonuclease
RNP ribonucleoprotein
SDS sodium dodecyl sulfate
snRNP small nuclear RNP
TEM transmission electron microscopy
TMV-MP tobacco mosaic virus movement protein
tubulin S subtilisin-treated tubulin
UTP uridine triphosphate
YB-1 Y-box binding protein 1

SUMMARY

YB-1 is a major regulator of gene expression in eukaryotic cells. In addition to its role in transcription, YB-1 plays a key role in translation and stabilization of mRNAs. We identify several novels YB-1 protein partners by affinity chromatography of different tissue extracts. We observed that YB-1 interacts with tubulin and microtubules and stimulates microtubule assembly *in vitro*. Microtubules assembled in the presence of YB-1 exhibited a normal single wall ultrastructure where YB-1 probably coats the outer microtubule wall. Furthermore, we found that YB-1 also promotes the assembly of MAPs-tubulin and subtilisin-treated tubulin. Additionally, we demonstrated that tubulin interferes with mRNA:YB-1 complexes. These results suggest that YB-1 may regulate microtubule assembly *in vivo* and that its interaction with tubulin may contribute to the control of mRNA translation.

The translational status of mRNPs *in vivo* depends on amount of YB-1 associated with mRNA. We show here that at low YB-1:mRNA ratios mRNP complexes possess an incompact structures, whereas saturated mRNPs are compact. This structural change corresponds to translation inhibition when mRNA moves from polysomal (translatable) to free (untranslatable) mRNPs. Saturated mRNPs bind to microtubules via protein:protein interactions and tend to self-aggregate on microtubule surface. This property could contribute to stress granule formation, mRNPs traffic and localization of translation apparatus within cytoplasm.

Finally, the facilitated diffusion model was developed to explain enhancement of microtubule assembly by positively charged natural polyamines in living cells.

Altogether our data contribute to the understanding of fundamental biological processes.

A. INTRODUCTION

A.1. Y-BOX PROTEINS

Numerous living organisms from bacteria to human contain members of the highly conserved “cold-shock” protein superfamily. These proteins act as transcription/translation factors and orchestrate processes of gene expression.

The human protein YB-1 was the first described member of the vertebrate “cold-shock” proteins. It has been identified by several independent groups seeking for trans-acting factors specifically interacting with different types of DNA promoters.

The first isolation of YB-1 gene resulted from the probing of a phage λgt11 expression library with double-stranded labeled oligonucleotides containing the Y-box sequence (5’ ATTGG 3’) found in major histocompatibility complex class II genes (Didier et al., 1988). This hybridization experiment revealed several positive clones that were identical. The cDNA of these clones contained about 1.5 kbases and encoded a protein with a molecular weight of 35.4 kDa, designated as YB-1 (Y-box binding protein 1). YB-1 displayed high level of specificity to DNA promoter sequences that highlighted its possible role in transcriptional regulation of Y-box containing genes. At the same time Sakura and colleagues (Sakura et al., 1988) using cDNA library from human placenta identified two proteins named DbpA and DbpB that displayed properties similar to YB-1. These proteins were specifically bound to DNA probes containing human epidermal growth factor (EGF) receptor enhancer or the human c-erbB-2 promoter. Similarly, rat liver phage expression library was probed by Rous sarcoma virus long terminal repeat enhancer (Ozer et al., 1990). A cDNA encoding a protein with enhancer binding activity was isolated and the corresponding protein (EFI_A) was overexpressed in *E. coli*. Analysis of the aminoacid composition of this protein revealed that EFI_A displays 97% homology with DbpB and YB-1. The recombinant EFI_A expressed in bacteria and the native protein purified from nuclear extracts of chicken embryos had the same molecular weight and discriminated between poly(dI):poly(dC) and specific promoter sequences, as YB-1. In addition, two cDNAs from Xenopus laevis library encoding Y-box proteins were isolated (Tafuri and Wolffe, 1990). The two proteins called FRGY1 and FRGY2 (frog Y-box germ proteins 1 and 2) show homology to YB-1 and a strong preference for Y-box containing sequences. These two proteins were expressed and purified

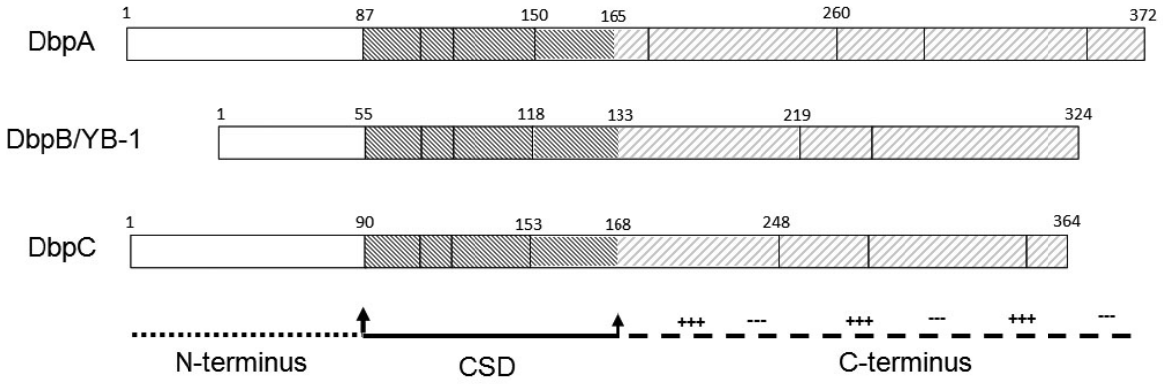
from *E. coli*. Pure proteins were found to stimulate transcription of oocytes-specific genes in Xenopus eggs extracts. Northern blot analysis of total RNA from Xenopus oocytes revealed that FRGY1 and FRGY2 are highly abundant in oocytes of stages I and II. However, at later stages of frog ontogenesis, FRGY2 mRNA disappeared totally and the level of FRGY1 mRNA declined significantly. Finally, in mature organisms FRGY1 is expressed in all tissues examined, while FRGY2 displayed a germ-cell specific pattern of expression. Together, all these pioneer works defined Y-box proteins as a specific trans-acting transcription factors acting in germ and somatic cells.

The human genome contains three different Y-box genes: DbpA, YB-1 (DbpB) and YB-2 (DbpC) located on chromosomes 12, 1 and 17 respectively. The human Y-box genes lack typical eukaryotic regulatory sequences, such as TATA and CCAAT boxes. They contain instead E-boxes, CG- and GATA motifs required for transcription.

The human Y-box proteins comprise three structural domains: the N-terminal, the central and the C-terminal domains. The N-terminal domain is always encoded by a single exon, whereas the number of exons which encode C-terminal domain varies from three to five. Surprisingly, the relatively small central part of Y-box proteins, containing only 78 amino acids is encoded by four exons (Figure 1, A). The central domain is located between aminoacid residues 55 and 132 (YB-1 numbering). This part of YB-1 shares 93% aminoacid sequence identity with other human Y-box proteins. The N-terminal and C-terminal parts of YB-1 are significantly less conserved and possess only 24% and 39% of identity respectively with corresponding domains of DbpA and DbpC.

It is worthy to note that human, rabbit and mouse YB-1 proteins are highly conserved and nearly identical. However, human DbpA, YB-1 and DbpC are significantly different (Figure 1, B). Most probably, the three human genes diverged during the course of evolutional development and now serve for different cellular functions and may replace each other during ontogenesis.

A:



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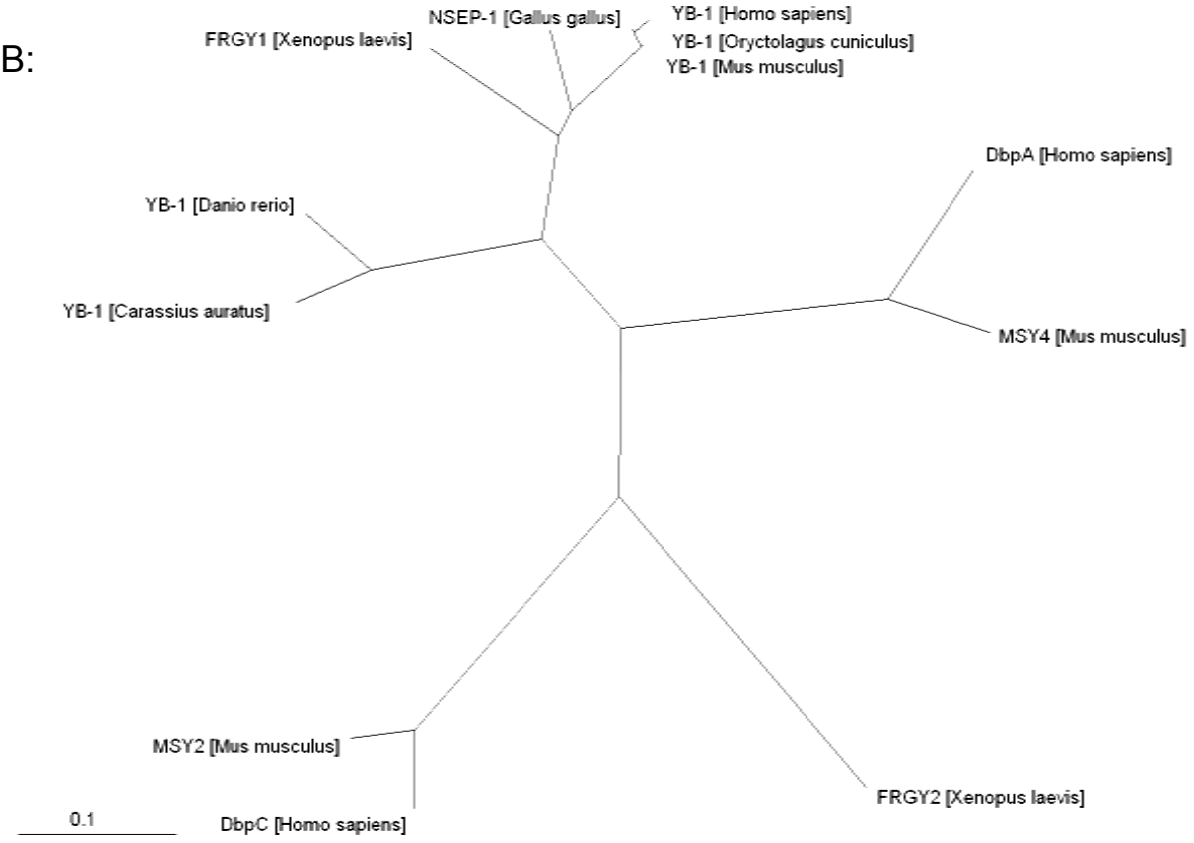

Figure 1: A: Shematic representation of exon composition of human Y-box proteins. Exons are boxed, numbers indicate bordering amino acids. Interestingly, the fifth exon of Y-box proteins encodes both a part of the central domain and of the C-terminal domain. B: Phylogenetic tree of vertebrate Y-box proteins. The lengths of branches correspond to relative distance between proteins. The bar below the graph represents 10% difference in the primary sequence.

A.1.1. Human YB-1 primary structure

The primary structure of YB-1 contains 324 amino acid residues and is highly enriched in arginine (12%), glycine (12%) and proline (10.8%). YB-1 possess an isoelectric point of 9.8 and is highly positively charged at neutral pH. Surprisingly, YB-1 migrates with an apparent molecular weight of 47 kDa on SDS-PAGE (Evdokimova et al., 1995), that could be explained by its high content of positively charged aminoacids (17% of total amino acids). The N-terminal domain (alanin-prolin rich domain) consists of the 54 aminoacid residues. It contains high number of alanine (22.2%), glycine (18,5%), proline (14.8%) and serine (11.1%). Central domain of YB-1 (cold-shock domain) contains 78 aminoacid residues (from 55 to 132) and possesses a more dispersed aminoacid distribution. It is only slightly enriched in glycine (12%) and valine (15%). Finally, the C-terminal domain comprising 192 aminoacid residues (from 133 to 324) is enriched in arginine (18.3%) and proline (12.6%). YB-1 possesses putative sites for phosphorylation, N-glycosilation, nuclear localization and cytoplasmic retention signals (Wu et al., 2007). The abundance of proline in its N-terminal and C-terminal domains suggests an absence of stable tertiary structures. The central domain of YB-1 appeared more structured and the secondary structure predictions using different algorithms indicated that the majority of the aminoacid residues form structured regions (α-helix or β-sheet). Contrary, the N-terminal and C-terminal domains are predicted to form large coiled regions (Figure 2).

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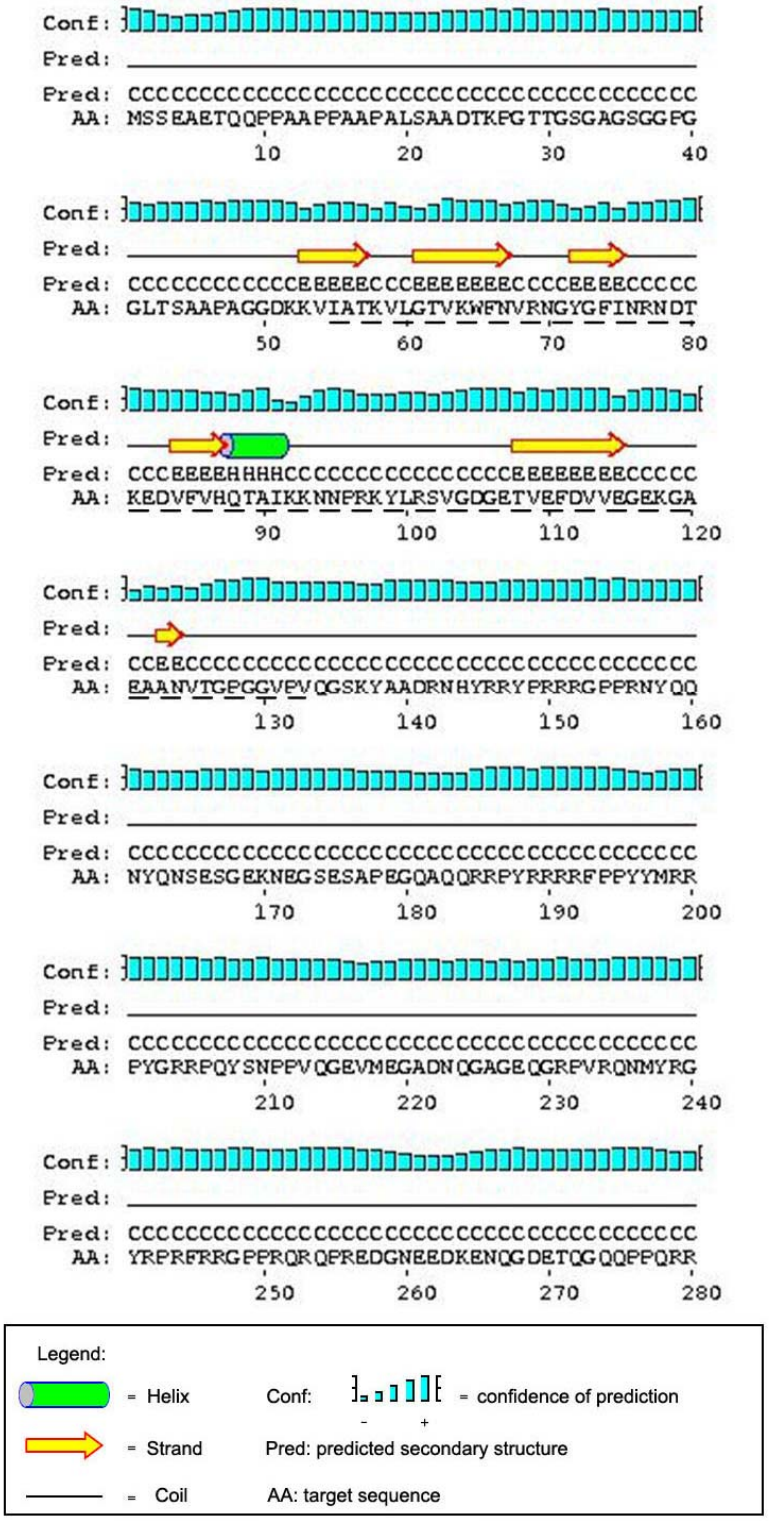


Figure 2: Secondary structure prediction for YB-1 using PredictProtein tool (Rost et al., 2004). Aminoacid residues of the «cold-shock domain» are underlined with dashed line.

A.1.2. Structure and functions of «cold-shock domain»

As stated above, the “cold-shock” domain of YB-1 is highly conserved within vertebrates, whereas distal domains are less conserved. Soon after the discovery of the cold-induced adaptation process in bacteria, sequences sharing partial homology with YB-1 were found in the bacterial proteins responsible for cold-shock adaptation (Csps) (Wolffe et al., 1992). These proteins displayed 43% identity to cold-shock domain of vertebrate Y-box proteins. Bacterial Csps are small proteins with molecular mass of 7.4 kDa actively expressed during the acclimation phase in bacteria observed after fast shift of bacterial culture from 37°C to 15°C. After the temperature shift, cells stop to grow for 4 hours, but overproduce many cold-shock induced proteins. During this lag-period, the concentration of cold-shock proteins reaches 100 μM, which represents more than 10% of the total cell proteins (Horn et al., 2007). Interestingly, the cold-shock proteins mRNAs are permanently presents in cells, but are rapidly degraded by cellular RNases at physiological temperatures. This indicates that the expression of cold-shock proteins is regulated at the post-transcriptional level, rather than usual transcriptional regulation observed for most of bacterial genes. The first described member of cold-shock family was CspA (cold-shock protein A) from *E. coli* (Goldstein et al., 1990). Eight other members of Csp proteins were identified since that time named from CspB to CspI. Interestingly, only CspA, CspB, CspG and CspI are cold-inducible, whereas CspD and CspE are expressed at temperature of optimal growth (Horn *et al.*, 2007). At low temperatures, cold shock proteins bind to and melt RNA secondary structure and this enhances the rate of translation (Graumann and Marahiel, 1998). Interaction of CspA with nucleic acids was characterized by numerous *in vitro* assays. CspA binds to RNA longer than 74 bases with poor sequence preference. The binding to RNA is cooperative above a minimal concentration of CspA of about $2*10^{-5}$ M (Jiang et al., 1997). Upon binding, CspA melts secondary structure of RNA and enhances its sensitivity to Rnases A and T1 *in vitro*. Most probably, *in vivo,* CspA acts together with DEAD-box helicases and facilitates ribosomal movement along mRNA. In addition, cold-shock proteins improve transcription elongation and act as transcription antiterminators at low temperatures (Phadtare et al., 2002). In agreement with this later proposal, mutations in CspE that diminish transcription antitermination activity also reduce its nucleic acid melting activity. Cold-shock domain is not a stable structure at room temperature

even in absence of denaturant (Schindler et al., 1999). Indeed, melting points of proteins from mesophilic bacteria are about of 50-60 °C which is unusually low. As a result, the affinity of cold-shock proteins to nucleic acids is highly temperature sensitive. The intrinsic thermal instability of cold shock domain helps to dissociate Csps from mRNA at optimal growth temperatures.

Soon after the discovery of cold-shock proteins, their structures from different sources were resolved by X-ray crystallography and NMR in solution (Schnuchel et al., 1993;Schindelin et al., 1994). Both methods revealed that the structure of cold-shock proteins is similar to oligonucleotide/oligosaccharide-binding fold, found in staphylococcal nuclease, yeast asp-tRNA synthetase and initiation factors IF1 and eIF2α (Murzin, 1993). CspA presents a β-barrel structure with a diameter of 1.5 nm formed by five antiparallel β-sheets (Figure 3). The structures of CspA and the CSD from YB-1 are mostly similar, except that the N-terminal part and the loop connecting β3 and β4 strands (L_{34}) are much longer in the case of CSD. The functional significance of such differences is not understood, although they may play a role in the proper orientation of Y-box proteins on DNA promoters (Kloks et al., 2002).

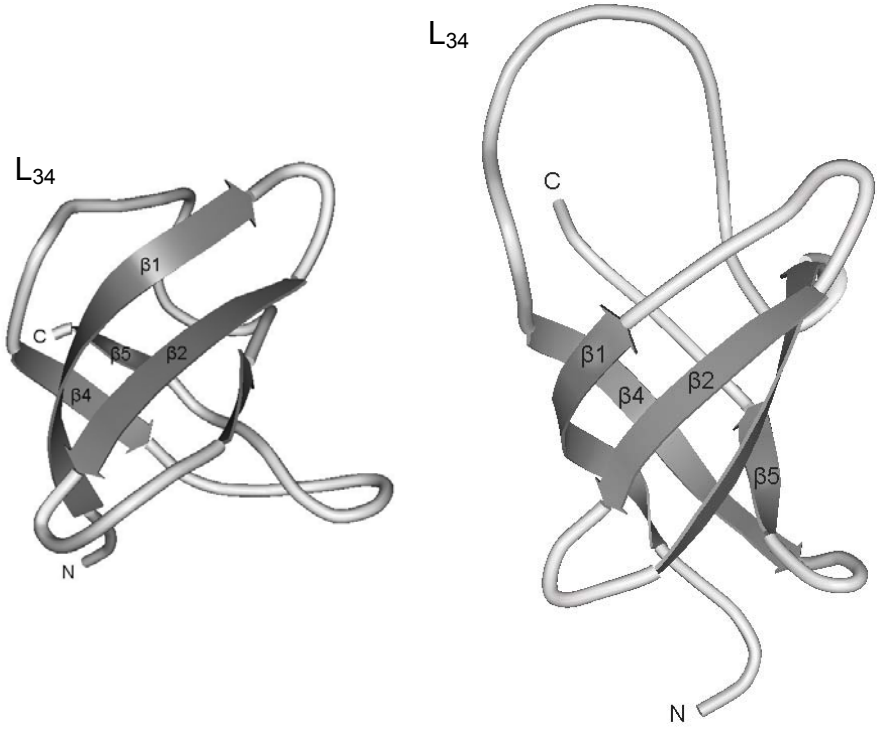


Figure 3: Structures of CspA from *E. coli* (left, PDB ID: 1MJC) and the «cold-shock» domain of human YB-1 (right, PDB ID: 1H95). Pictures were made with Protein Workshop software (Moreland et al., 2005).

The detailed analysis of the cold-shock domain revealed that it contains two conserved aminoacid motifs: RNP-1 (K/R-G-F/Y-G/A-F-V/I-X-F/Y) and RNP-2 (L/I-F/Y-V/I-G/K-N/G-L), located on the β-2 and β-3 strands respectively (Landsman, 1992;Kloks *et al.*, 2002). The RNP-1 motif consists of eight aminoacid residues with a hydrophobic core. This motif have been found in the sequences of hnRNP, snRNP, poly (A) binding, polypyrimidine tract binding and other nucleic acid binding protein families (Landsman, 1992). Probably, the RNP-1 motif provides a general nucleic acid binding properties. As mentioned, both RNP-1 and RNP-2 motifs are enriched in hydrophobic amino acids. Usually aromatic and hydrophobic amino acid residues are buried inside compact protein globule and very rarely can be found on the protein surface. Interestingly, in the case of CSDs the aromatic aminoacids of the RNP-1 and RNP-2 motifs are exposed to the protein surface. Unexpectedly, the replacement of these hydrophobic amino acids by alanine destabilized the protein (Schindler et al., 1998). Corroborative analysis revealed that all side chains of positive and aromatic aminoacids are important for the creation of the surface acting as nucleic acids binding site (Schroder et al., 1995). The RNP-1 and RNP-2 motifs are in close contact at the surface of protein globule together and form compact aromatic patch. Presumably, the aromatic residues bind to nucleic acids by stacking and play a major role in the recognition of specific nucleotide sequences, while the basic aminoacids bind to external sugar-phosphate backbone of RNA and DNA.

A.1.3. Structure and functions of N- and C-terminal domains of YB-1

Unfortunately, less is known about the structure of the N-terminal and C-terminal domains of YB-1. Most probably, the N-terminal domain doesn't play any role in nucleic acid recognition, however it could stabilize cold-shock domain against temperature unfolding (Kloks *et al.*, 2002). Although the function of the N-terminus is still not clear, some proposals based on *in vitro* data have been made. Surprisingly, the N-terminal extremity of YB-1 shares some homology with myosin, actobindin and tropomyosin. It has been shown that the N-terminal domain of YB-1 interacts with actin and co-sediments with actin filaments *in vitro*. Moreover, YB-1 promotes the interaction of mRNA with actin filament, depending on the YB-1/mRNA ratio. On this basis, it has been proposed that YB-1 can anchor mRNP on actin cytoskeleton and

thus participate in cellular localization of mRNA (Ruzanov et al., 1999). Recently, the N-terminal domain has been identified as a site for interaction with the tumor suppressor factor p53 (Okamoto et al., 2000).

The C-terminal domain of YB-1 proteins contains several positive aminoacid clusters, which alternate with negatively charged stretches. This domain plays a major role in the binding to nucleic acids. Residues 278-292 of YB-1 share 67% of homology with residues 10-24 of caspian sturgeon protamine (Didier *et al.*, 1988). This small arginine-rich protein binds strongly to nucleic acids and participates in DNA condensation in sperm head. The C-terminal domain of YB-1 is enriched in arginine, highly positively charged, and also demonstrates similar strong binding to nucleic acids. Notably, the C-terminal domain contains repetition of YRR motif, the two or three arginine residues recognize the phosphate backbone of nucleic acids, while the adjacent tyrosine residue might intercalate between bases and increase the affinity for nucleic acids. Other cellular proteins, like proteins of heterogenous nuclear RNP, splicing and polyadenylation factors also contain repetitions of basic RNA-recognition motifs, like RGG and KH and SR boxes (Dreyfuss et al., 2002b). As a rule, these boxes modulate functions of RNA-binding domain(s), including CSD, and serve for protein-protein interaction and protein localization. The importance of the C-terminal domain of Y-box proteins for the interaction with RNA was evidenced by fragmentation of FRGY-2 (Ladomery and Sommerville, 1994a). Indeed, disruption of cold-shock domain had only a slight effect on protein RNA-binding properties (Ladomery and Sommerville, 1994b). This suggested that the C-terminal domains play a major role in nucleic acid recognition. Additionally, fragmentation of the protein with formic acid, which resulted in multiple cleavages in C-terminal region of protein, revealed that the positively charged clusters bind to RNA independently of each other. These experiments suggest that each of YRR containing clusters additively increases the affinity of whole protein for nucleic acids. Y-box proteins bind very avidly to RNA with dissociation constants of 10^{-8}-10^{-9} M, while isolated cold-shock domain binds to RNA with dissociation constant of 10^{-5}- 10^{-6} M. Together these results indicate an exceptional significance of the C-terminal domain for nucleic acid binding (Kloks *et al.*, 2002). Another well described function for the C-terminus is its ability to mediate protein-protein interaction. This domain contains alternative charged clusters that act as “charge zippers” and promote self-oligomerization of Y-

box proteins (Tafuri and Wolffe, 1992;Skabkin et al., 2004). Finally, the C-terminal domain contains sites for binding to hnRNP K (Shnyreva et al., 2000), p53 (Okamoto *et al.*, 2000), and iron-regulatory protein (IRP-2) (Ashizuka et al., 2002) and together with CSD forms sites for interaction with transcriptional factors (Ansari et al., 1999;Safak et al., 1999).

A.2. ROLE OF MESSENGER-RNA-BINDING PROTEINS IN THE REGULATION OF GENE EXPRESSION

Soon after the discovery of messenger RNA in 1961, it appeared that nascent RNA exists in complex with cellular proteins in the form of ribonucleoprotein particles (RNP) (Spirin and Nemer, 1965). The association of proteins with newly synthesized transcripts starts at the very beginning of transcription; even if the synthesis of pre-mRNA is not accomplished. These proteins, which serve as nannies for transcript, prevent abortive synthesis and help RNA polymerase II to complete synthesis correctly. During RNA synthesis, the long C-terminal tail of RNA polymerase serves as a bait for numerous capping, splicing and polyadenylation factors, so at the end of transcription RNA is fully coated by proteins (Maniatis and Reed, 2002). These proteins define future destiny of nascent RNA and eliminate defective transcripts. After the end of splicing, RNA changes its clothes and a group of nuclear export factors helps RNA to migrate from nucleus to cytoplasm. Finally, in the cytoplasm, mature mRNA associates with translational factors that assist protein synthesis (Dreyfuss et al., 2002a). Two major proteins with molecular masses of 70 kDa (p70) and 52 kDa (p50) were found to be components of cytoplasmic mRNP from different sources (Blobel, 1972;Ovchinnikov et al., 1978b;Minich et al., 1993;Soop et al., 2003). Both these proteins were tightly bound to mRNA even in the presence of high concentration of salt and absence of magnesium that unambiguously indicated their specificity for mRNP formation. Treatment of mRNP with ribonuclease revealed that p70 (PABP) is bound to poly (A) tail, while p50 (subsequently recognized as YB-1) is distributed along all the RNA body. Since that time the properties of YB-1 and PABP were extensively investigated and for both proteins it has been shown that they participate in the processes of translation initiation, mRNA localization and stability. Recent studies revealed that the cytoplasmic mRNPs also contain DEAD-box RNA

helicases which rearrange structure of mRNP by modulating protein-protein and RNA-interactions (Ladomery et al., 1997;Nakamura et al., 2001;Nashchekin et al., 2006b). It was proposed that RNA helicases unwind secondary structure of mRNA, interact directly with YB-box proteins and promote their binding to mRNA (Nashchekin et al., 2006a). Thus, the synergism between the different protein components of mRNP could play an important role in regulation of mRNA translation status.

A.2.1. Structure and functions of PABP

In most cellular mRNAs, the open reading frame is bordered by 5' and 3' untranslated regions and, in addition, mRNAs contain long 3' poly(A) tail. Untranslated regions are encoded in the genomic DNA, while the poly(A) tail is not transcribed, but added via polyadenylation reaction. In eukaryotes, cleavage of pre-mRNA and polyadenylation are closely related, because both these reactions are performed by a single huge protein complex, that includes cleavage factor (CSPF), poly(A) polymerase and many additional factors. Typically, the resultant mRNA contains poly(A) tail of about 200-250 nucleotides in mammals and 70-90 residues in yeast (Mangus et al., 2003). Several homological cytoplasmic protein that interact with mRNA poly(A) tail were designated as poly(A) binding proteins (PABPs). There are four human PABPs: PABP, PABPC3, iPABP, PABPC5. All these proteins demonstrate sequence preference for poly(A) and have similar modular structure. PABPs consist of four repetition of RNA-binding domains (RBD), containing RNP-1 and RNP-2 motifs. The X-ray structure of a C-terminal truncated fragment PABP containing RRM1 and RRM2 has been resolved (Deo et al., 1999). This fragment possesses a globular structure, which consists of four antiparallel β-strands backed against two α-helices (Figure 4).

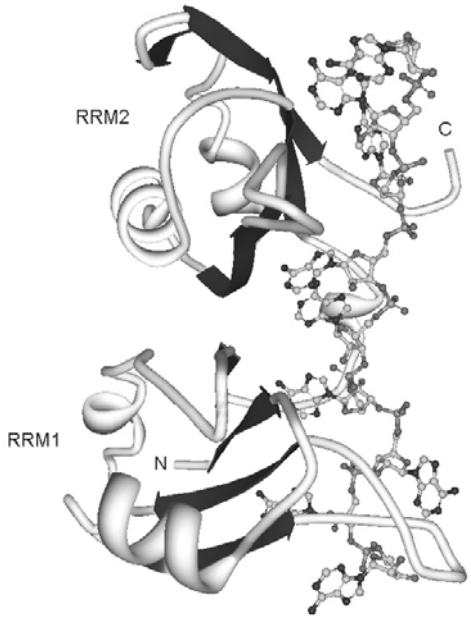


Figure 4: Structure of RRM 1-2 of PABP bound to poly (A) (PDB ID: 1CVJ). Picture was made with Protein Workshop software.

This type of fold, named Rossman fold, is typical for many cytoplasmic proteins, like nucleotide binding

proteins and enzymes. Conserved amino acid residues in the β-sheet recognize poly(A) nucleotide leaving α-helices free for protein-protein interaction (Cheng and Gallie, 2007). The C-terminal domain of PABP, composed of five α-helixes, contains sites for binding to eRF3, PAip1-2, Pbp1p and a viral RNA polymerase (Kozlov et al., 2001). This conserved domain contains 74 aminoacid residues and regulates PABP shuttling between cytoplasm and nucleus. Although the function of this shuttling is still unclear, it could be involved in nuclear export of mRNA. Since the first description of PABP by Gunter Blobel (Blobel, 1973), the interaction of poly(A) with PABP became one of the most known and well described among protein-nucleic acid interaction in the mRNP world. Poly(A) tail together with PABP acts as enhancer of translation and protects mRNA from degradation (Gorgoni and Gray, 2004). Positive effect of poly(A) tail on protein synthesis nowadays is always explained by the interaction between PABP and eIF4G proteins, which leads to mRNA cyclization. In addition, binding of PABP to 3' UTR of YB-1 mRNA significantly enhance its translation (Skabkina et al., 2005). This effect was explained by competition between PABP and YB-1 for interaction with specific A-rich sites. Interestingly, the C-terminal domain of PABP can interact with eRF3 and modulate translational termination and polypeptide release. This interaction leads to an increase in the pool of free 40S ribosomal subunits, facilitating multiple rounds of translation initiation on the same mRNA molecule.

A.2.2. Y-box proteins as a component of cytoplasmic mRNPs

As mentioned above, cytoplasmic mRNAs are engaged in complexes with cellular proteins that accompanies mRNA for its different function. The hypothesis that mRNA carries proteins necessary for translation has been proposed for the first time at the end of 70-th by Alexander Spirin (Spirin, 1978). Briefly, this concept proposes that translatable polyribosomal mRNPs are bound to initiation, elongation and termination factors, whereas silent mRNPs contain special proteins that mask mRNA. Accordingly, newly synthesized heterogeneous nuclear RNA is also associated with proteins responsible for splicing and polyadenylation. Moreover, this concept presumed that RNA species could also carry different enzymes implicated in the modification of translational factors.

Ribonucleoprotein particles purified from different natural sources share the same physicochemical properties. mRNPs isolated from the cytoplasm of fish embryos, sea urchin eggs and rabbit reticulocytes possess the same buoyant density of 1.4 g/cm^3 for masked mRNPs and 1.45 g/cm^3 for polyribosomal mRNPs (Ovchinnikov et al., 1978a;Minich *et al.*, 1993;Ovchinnikov et al., 2001). Accordingly, calculated protein/mRNA weight ratio for free mRNPs is three and for polysomal mRNPs is two. All types of cytoplasmic mRNPs were found to be extremely sensitive to endoribonuclease treatment and resistant against magnesium depletion that highlighted differences in structures of mRNPs and ribosomes.

The repression of translation is a common strategy to regulate gene expression at early stages of development. The best known and one of the most explored model system to study translational regulation is Xenopus oocytes. During the amphibian oogenesis, oocytes transcribe and accumulate huge pool of mRNAs most of which being repressed. Due to their relatively large size, oocytes are suitable for microinjection experiments. Surprisingly, exogenous mRNA injected into oocyte cytoplasm is efficiently translated, despite the repression of endogenous mRNAs. This result indicated that endogenous mRNAs are somehow associated with cellular factors that repress translation. Biochemical analysis of stored mRNPs revealed that they possess a buoyant density of 1.4 g/cm^3 and contain two major phosphoproteins of 60 kDa and 56 kDa that were identified as FRGY2 (Kick et al., 1987a). Repression of translation of endogenous mRNAs is relieved in response to developmental stimuli, such as progesterone treatment that stimulates oocyte maturation. It has been demonstrated that progesterone treatment activates cellular phosphatases and can be reversed by addition of okadaic acid. In addition, it was observed that inhibitors of casein kinase II, such as DRB or quercetin activated translation of silent mRNA. Finally, experiments *in vitro* provided direct proofs that FRGY2 protein loses its affinity for mRNA after dephosphorylation with protein phosphatase A (Kick et al., 1987b). Taken together these data suggest that maturation stimuli activate cellular phosphatase(s) that dephosphorylate FRGY2 and lead to dissociation of FRGY2 from mRNPs. Dephosphorylation of FRGY2 activates translation of silent mRNA, whereas protein phosphorylation by casein kinase II causes global translation repression (Braddock et al., 1994).

Another protein able to form cytoplasmic RNPs with properties similar to that of FRGY2 was found in rabbit reticulocyte extracts (Minich *et al.*, 1993). The major RNP protein from reticulocyte possesses a molecular weight of 50 kDa and was designated as p50. Lately, p50 was recognized as a member of the Y-box family and renamed as rabbit YB-1 (Evdokimova *et al.*, 1995). This protein was found in both polysomal and masked RNPs, but the concentration of p50 varied in function of the translational status of RNPs. Indeed, the amount of p50 in masked RNPs was twice higher than in polysomal RNPs that indicated a repressive effect of p50 on mRNA translation. Recent observations elucidated the molecular mechanism of translation inhibition by YB-1 (Nekrasov et al., 2003). Translation inhibition by YB-1 was accompanied with the displacement of translation initiation factors eIF4E and eIF4G from mRNA. The competition between YB-1 and translational factors for the binding to mRNA blocks the first step of cap-dependent translation initiation. Furthermore, it was found that the N-terminal part of YB-1 is not able to repress translation, whereas the C-terminal moiety produces an inhibitory effect. This strongly suggests that repression of translation is provided by a non-specific affinity of arginine-rich C-terminus for mRNA.

A.2.3. Role of YB-1 in structural organization of cytoplasmic mRNPs

A detailed biochemical characterization of rabbit YB-1 revealed that the purified protein in physiological conditions exists in the form of multimeric globule with a mean molecular mass of 800 kDa and a sedimentation coefficient of 18S (Evdokimova *et al.*, 1995;Skabkin *et al.*, 2004). Rabbit YB-1 and FRGY2 form mRNP complexes that possess a buoyant density of 1.4 g/cm^3, which is similar to the density of natural non-translated mRNPs (Matsumoto et al., 2003;Skabkin *et al.*, 2004). The AFM and EM analyses of YB-1:mRNA complexes revealed that they contain several globular particles with a height of 8-10 nm and a diameter 30-40 nm each. The number of particles per mRNP depends on mRNA length, usually one particle occupies about 600-700 nucleotides. Most probably, each particle is formed by twenty molecules of YB-1 and a fragment of mRNA with a length of 700 nt packed on the surface of this globule. The reconstituted YB-1:mRNA complexes possess thus a “beads-on-string” structure that strikingly resembles the structure of hnRNP

(Samarina et al., 1968;Conway et al., 1988). It is worthy to note that the sedimentation coefficient of mRNPs and dimensions of protein globules within them increase gradually with the protein:mRNA ratio. mRNP complexes formed at low YB-1:mRNA ratio are significantly more flat and possess other type of RNA compaction. This indicates the existence of structural rearrangements of mRNP provoked by the addition of YB-1. Relative flatness of complexes that contains small amounts of YB-1 is in agreement with the binding of YB-1 to RNA in a monomeric form. As mentioned above, YB-1 interacts with RNA via its «cold-shock» and C-terminal domains that possess different RNA-binding properties. Interestingly, an increase of the protein:RNA ratio provokes partial displacement of the C-terminal domain from mRNA. It has been proposed that the displaced C-termini becomes free to interact with one another which promotes multimerization of YB-1 on mRNA. It seems that the multimeric state of YB-1 inside RNP globules at high protein:mRNA ratio is similar to that of the free protein. These structural observations support the idea that YB-1 is indeed involved in the global regulation of mRNA translation (Figure 5).

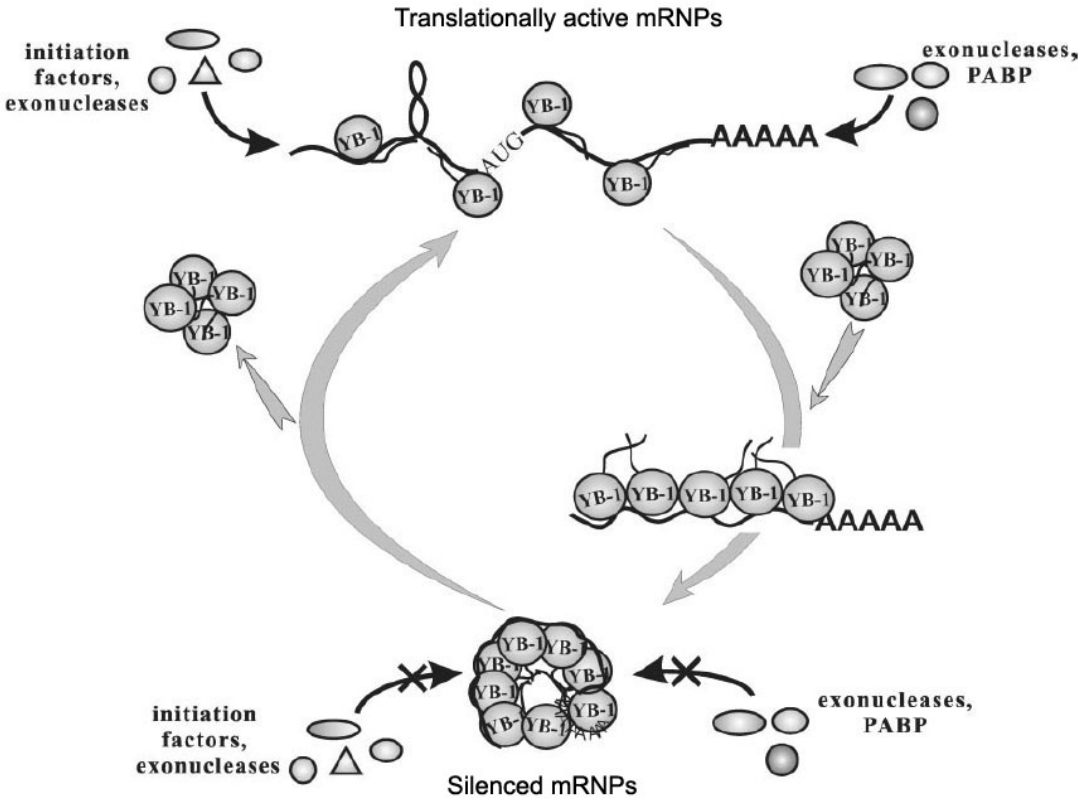


Figure 5: Hypothesis for the translation regulation by Y-box proteins. From Skabkin et al., 2004.

The activation of silent mRNA corresponds to a decrease of the YB-1:mRNA base ratio which leads to a better accessibility of mRNA for translational factors and nucleases. On the other hand, addition of extra YB-1 provokes mRNPs compaction, inhibits mRNA translation and masks it from interaction with translational factors and nucleases.

Despite the homology between YB-1 and FRGY2, the molecular mechanisms of their displacement from mRNA are different. As mentioned above, the affinity of FRGY2 for mRNA is regulated by phosphorylation with casein kinase II. Opposite to FRGY2, YB-1 is phosphorylated at Serine 102 by the serine/threonine protein kinase Akt which is implicated in the processes of cellular proliferation, growth and survival (Sutherland et al., 2005;Evdokimova et al., 2006;Bader and Vogt, 2008a). Activation of Akt kinase *in vivo* could be induced by treatment of cells with growth factors, for example, insulin-growth factor (IGF-1). Activated form of Akt kinase also accompanies breast and lung cancer and is correlated with poor prognosis (Vivanco and Sawyers, 2002). It has been shown that Akt activation relieves translational block of silenced mRNA species including transcription, growth factors and other proteins associated with cancer (Evdokimova *et al.*, 2006). Moreover, Akt kinase was found in RNP complexes that could indicate immediate transmission of growth signal to YB-1 and translation activation. It was found however, that Akt phosphorylation does not change the overall affinity of YB-1 to mRNA, but only specifically removes YB-1 from proximity of capped 5' mRNA terminus. As a consequence, Akt activates only cap-dependent translation and does not produce any effect on IRES-dependent translation. In agreement with this observation, it has been proposed that YB-1 may serve as a tumor suppressor factor that blocks translation initiation of specific mRNA implicated in cell growth (Evdokimova *et al.*, 2006;Wu *et al.*, 2007).

A.2.4. Stress granules as temporal sites for translation regulation

Some cellular stresses, such as arsenite-induced oxidative stress, heat shock and viral infection provokes formation of cytoplasmic foci containing aggregates of mRNAs. Formation of these mRNA-containing structures is followed by translation arrest, rapid disassembly of polysomes and package of mRNA intro stress granules (Kedersha et al., 2005;Kedersha and Anderson, 2007). Cytoplasmic stress granules

are huge complexes with diameter above micron that allowed their direct visualization under optical microscope. Regardless of stress granules size, cell needs only 15-30 min to aggregate the majority of mRNAs into these structures with the remarkable exception of mRNA encoding heat-shock proteins (Anderson and Kedersha, 2006). Analysis of stress granule composition revealed that they contain initiation factors eIF2, eIF3, eIF4F and 40S ribosomal subunit. In that type of complexes small ribosomal subunit is stalled near the 5' end of mRNA and unable to initiate protein synthesis. In most of the cases, stress-induced translation arrest occurs in parallel with the phosphorylation of eIF2 by several cellular kinases. Depending on stress stimuli, the serine 51 in the alpha subunit of eIF2 can be phosphorylated by PKR (viral stress), PERK (endoplasmic reticulum stress), GCN2 (aminoacid starvation) and HRI (arsenite). Phosphorylated eIF2 forms stable complex with nucleotide-exchange factor eIF2B and inactivates it. Subsequently, deprivation of eIF2B leads to accumulation of eIF2-GDP complexes and translation shuts-off. Another possible target for stress-signals is helicase eIF4A that unwind 5' UTR and stimulates movement of small ribosomal subunit along mRNA. Helicase activity of eIF4A can be inhibited by natural drugs as pateamine A and hippuristanol that induce stress granule formation independently of eIF2 phosphorylation. Finally, puromycin that mimics 3' end of aminoacyl-tRNA and promotes abortive polypeptide release also initiates assembly of stress granules. Thus, accumulation of RNP in stress granules can be provoked by different agents that block translation initiation at early stages. In addition to classical translation factors, stress granules contain pool of proteins that regulate different aspects of mRNA metabolism. These proteins include major RNP components, like PABP and YB-1, together with nucleases, helicases and other regulators of mRNA biogenesis. Despite the fact that both stress granules and untranslated RNP serve for storage of silenced mRNA, stress granules are not stable structures, and this feature makes their biochemical isolation quite difficult. Under stress conditions, cell remodels, modifies and sorts mRNP content of stress granules continuously, whereas masked mRNP are stable structures that can be found even in optimal growth conditions (Anderson and Kedersha, 2008a). Many questions concerning the composition and modifications of stress granules protein moiety remains to be answered since they have been described only eight years ago, whereas masked RNP are known more than forty years. Most probably, different proteins may trigger stress granule formation and post-translational modifications of

central RNP components. In this context, PABP and YB-1 could play specific roles in these processes.

A.3. STRUCTURE AND FUNCTIONS OF TUBULIN AND MICROTUBULES

Microtubules are major components of the cytoskeleton of all eukaryotic cells. These polymers participate to the intracellular organization and maintenance of cellular integrity. They are essential for cellular life owing to their implication in numerous cellular processes, like determination of cellular shape, position of organelles, cellular motility, movement of intracellular organelles and formation of the mitotic spindle during cell division. Microtubules are cylindrical structures composed of tubulin subunits (α and β), non-covalently bound together. Microtubules can rapidly assemble and disassemble in response to different cellular stimuli. The inherent microtubule dynamicity is provided by hydrolysis of GTP and closely related with microtubule functions. Tubulin is a dimer of α/β subunits each of which having a molecular mass about 50 kDa. The atomic three dimensional structure of tubulin heterodimer was studied by independent groups (Nogales et al., 1998;Gigant et al., 2000;Schlieper et al., 2005). Initially, structural data were obtained by electron diffraction performed on zinc-induced taxol stabilized tubulin sheets (Figure 6)

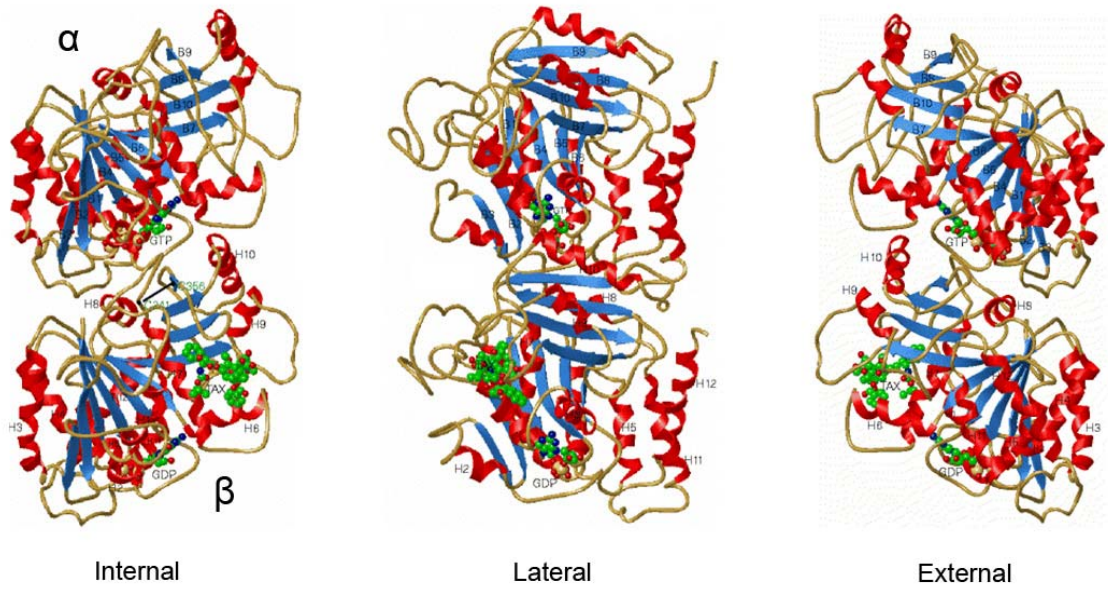


Figure 6: Structure of the α/β tubulin heterodimer. Views from the inner and lateral sides view and from outside of the microtubule are shown. The elements of the secondary structure are shown in different colors: α-helices in red, β-strands in blue and coiled regions in yellow. The nucleotides that bound to both subunits and taxol associated with β-tubulin are shown in green. Modified from Nogales et al., 1998.

(Nogales *et al.*, 1998). X-ray diffraction experiments performed on stathmin:tubulin complexes provided the best resolution available to date, 3.5 Å (Ravelli et al., 2004) which is, however, still not sufficient to understand the relation between structure and activity of tubulin in details.

Both subunits consist of a core formed with ten β-sheets surrounded by 12 α-helices. The most significant differences between subunits are situated in loop M (microtubule loop), which participates to lateral interaction between protofilaments. Despite common structural fold, α and β subunits display only 40% sequence identity. The most conserved domains are located in regions involved in lateral and longitudinal contacts between subunits. The most variable domains are located in the C-termini of subunits that undergo post-translational modifications and are involved in protein-protein interaction. The tubulin heterodimer contains non-exchangeable and exchangeable nucleotide binding sites, located on α- and β-subunits respectively. Due to these differences the α-subunit is always bound to GTP, whereas β subunit can also be loaded with GDP.

Tubulin consists of three domains that serve for different functions. The N-terminal domain, including amino-acids 1-205, contains the GTP-binding site. The intermediate domain is able to interact with different drugs, while the C-terminal domain interacts with different protein partners.

Cellular microtubules appear as hollow cylinders of about 25 nm in diameter. The cylinders within cells are formed by the lateral association of 13 protofilaments in mean. Each protofilaments is a string-like structure, which consists of tubulin subunits that are arranged in a head-to-tail orientation, a property that confers a polarity to microtubules.

In vivo, microtubules consist mainly of 13 protofilaments, while *in vitro* their number can vary from 9 to 16. Two models were proposed to describe lateral interaction between protofilaments. In the first model A (A-lattice) α-subunits from one protofilaments interact with β-subunits from the other and vice versa, in the other model (B-lattice) protofilaments are bound together by interaction between the same subunits (α interact with α and β with β). Regardless the fact that microtubules *in vitro* and *in vivo* could present both types of organization, the second structural type (B lattice) is more frequently found in living cells. In this model, interaction between

protofilaments results in helical pattern of monomers on the surface of the microtubule (Figure 7).

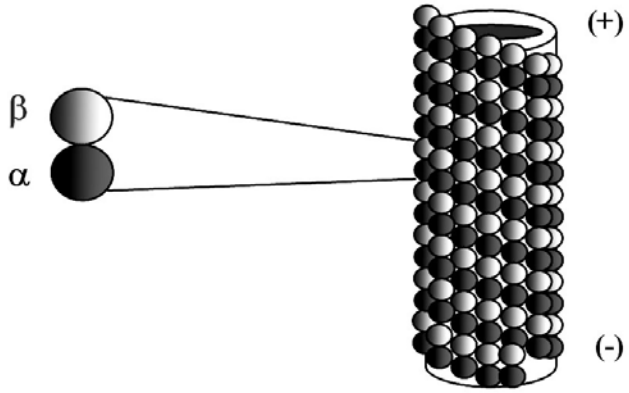


Figure 7: Schematic representation of B-lattice microtubule organization. Microtubule polarity indicates that β-subunits are exposed at «plus» end, whereas α-subunits on «minus» end of the microtubule.

In the case of microtubules made with 13 protofilaments, the height of one turn corresponds to 3 tubulin monomers. The “head-to-tail” orientation of αβ-tubulin in microtubules results in a different subunit exposition at their ends: α subunits are exposed to solvent at the minus end (-) and β at the plus end (+). Microtubule ends were named in this manner because “plus” end is more dynamic, than “minus” end (Desai and Mitchison, 1997). The polarity of microtubule ends was unambiguously demonstrated by several approaches. Antibodies specific for α-tubulin recognized exclusively “minus” end of microtubules, whereas GTP-coated fluorescent beads binds only to “plus” end (Mitchison, 1993;Fan et al., 1996).

A.3.1. Microtubule nucleation process

Nucleation corresponds to the first stage of tubulin polymerization. It involves the formation of tubulin oligomers that serve as nuclei for microtubule growth. Different models were proposed for *in vitro* nucleation. One model proposed that a nucleus consists of 6-12 tubulin dimers laterally brought together by spontaneous aggregation. Another model proposes that tubulin molecules are bound together via longitudinal interaction. However, nucleation *in vivo* was observed at specific places in the cytoplasm, known as microtubule-organizing centers. For many years,

centrosomes were described as the major cellular microtubule organization center. The centrosome is formed by two centrioles surrounded by the pericentriolar material matrix. Each centriole consists of a 500 nm long cylinder made of nine microtubule triplets. Due to the fact that duplicated centrosomes are placed at two cellular poles during mitosis, they were described as structures indispensables for the assembly of mitotic spindle. However, recent experiments revealed that even after disruption of centrosomes, cells can still assemble mitotic spindles (Mahoney et al., 2006). Moreover, numerous plant and fungi species lack centrioles. Nowadays, the conception of microtubule organizing centers assumes that new microtubules can nucleate on structures containing γ-tubulin (Luders and Stearns, 2007). This new member of the tubulin family was found as a component of a supramolecular complex containing eight other proteins. The γ-tubulin ring complex (γ-TURC) contains 13 molecules of γ-tubulin and appeared as a ring-shaped structure with a diameter of 25 nm (Zheng et al., 1995). Reasonably, such structural organization provides to this complex microtubule-nucleating properties. Two mechanisms of microtubule nucleation by γ-tubulin ring complex were proposed (Figure 8) (Job *et al.*, 2003). The “template model” states that ring complex serves as a template for microtubule growth and new tubulin subunits associates with it longitudinally. This model presumes that γ-tubulin molecules contact only with α-tubulin. Alternatively, the “protofilament model” describes ring complex as a primer for single protofilaments growth, where γ-tubulin can be in contact with both subunits (Raynaud-Messina and Merdes, 2007).

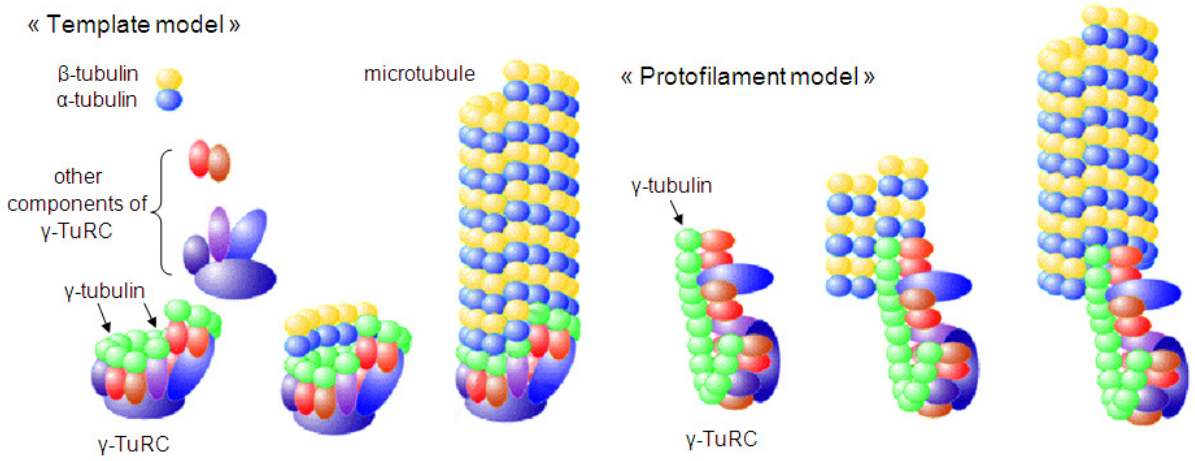


Figure 8: Two models of microtubule nucleation. Modified from Job et al., 2003.

A.3.2. Microtubule dynamics *in vitro*

As mentioned above, microtubules are highly dynamic structures. There are two models of microtubule dynamicity: the “treadmilling” and “dynamic instability” models. Treadmilling means that tubulin dissociate from one end of microtubule and associate with the other end. Dynamic instability describes changing between phases of growth and phases of rapid microtubule depolymerization. The transition between growth and depolymerization phases are called “catastrophes”, while transitions between depolymerization and growth are called “rescues”.

Treadmilling was the first concept to describe dynamic behavior of microtubules (Kirschner, 1980). Process of microtubule assembly *in vitro* can be separated into three phases: nucleation, elongation and steady-state phases when polymerization is already accomplished. At steady-state, treadmilling assumes that tubulin dissociate from “minus” end and associate with microtubule “plus” end. This implies that association/dissociation events of heterodimer at the microtubule ends do not change the length of microtubules, but result in movement or “treadmilling” of single heterodimer from “plus” to “minus” ends of microtubules.

The other mechanism, termed “dynamic instability”, was based on the observation of single microtubule by videomicroscopy (Mitchison and Kirschner, 1984;Walker et al., 1988c). It has been shown, that even at steady-state the length of single microtubule is not stable and varies with time. Thus, considering a single microtubule, it alternates between periods of growth and shrinkage, but the bulk of microtubules exhibits a steady-state. Instability of microtubules is a complex process, including reactions of tubulin polymerization and depolymerization (Desai *et al.*, 1997). Microtubule polymerization is a bimolecular reaction, dependent on concentrations of tubulin and microtubule ends, whereas depolymerization is a monomolecular reaction independent on tubulin concentration. Consequently, tubulin concentration determines rate of polymerization, but the rate of depolymerization is independent on tubulin concentration (Erickson and O'Brien, 1992;Flyvbjerg et al., 1996a). Increase in tubulin concentration increases polymerization rate and rescue frequency and decreases frequencies of catastrophe. In addition to rescue/catastrophe phases, microtubules sometimes loose dynamicity and stop cycles of polymerization/depolymerization (Waterman-Storer and Salmon, 1997). Mechanism

of such pausing is also not understood, although some destabilizing drugs increase probability of pausing on the “plus” ends. Together, tubulin dynamics appears thus as a very complex process, dependent on many parameters (Desai *et al.*, 1997;Janosi et al., 2002).

A.3.3. Structural changes associated with microtubule assembly

The incorporation of a tubulin inside polymeric lattice provokes the hydrolysis of GTP bound to E-site on β-subunit (Hyman et al., 1992). This hydrolysis results in changing of contacts between subunits that was revealed by different structural methods (Hyman et al., 1995;Muller-Reichert et al., 1998). Electronic microscopy of different intermediates of tubulin polymerization revealed that microtubules are always straight structures, while products of depolymerization present curved structures (Mandelkow et al., 1991). More recently, cryoelectron microscopy studies revealed that structures of polymerizing and depolymerizing ends are different. In conditions that favor polymerization, plus ends of microtubules were observed as long and flat protofilament sheet, whereas during depolymerization single protofilaments were peeled out from the microtubule ends (Chretien et al., 1999). Structural comparison of protofilaments from broken microtubules formed in presence of low-hydrolysable GTP analog GMPCPP revealed that they are significantly straighter, than GDP-containing protofilaments. Additional evidences for structural changes in heterodimer structure were provided by comparison of conformations of tubulin inside microtubule with that of unpolymerized tubulin in complex with stathmin (Gigant *et al.*, 2000;Ravelli *et al.*, 2004). The angle between two dimers inside microtubule was about 5 degrees, whereas the angle between two stathmin-bound dimers was around 12 degrees. These observations raised the idea that transition between GTP and GDP-bound tubulin states correlates with changing in curvature of tubulin. In other words, GTP-bound tubulin can accommodate straighter conformation, than GDP–bound dimer. Logically, dynamic behavior of microtubules was explained on basis of this structural data. It was proposed that in conditions that favor polymerization microtubule ends represent flat sheet that can eventually form cylinder. The flat lattice traps protofilaments in straight conformation and acts as a cap that stabilizes microtubule ends against disassembly. Cylindrical closure of the sheet increases

probability of catastrophe, because protofilaments can curve out from cylinder and provoke microtubule depolymerization. GTP cap structure formed by GTP tubulin is more rigid than the rest of microtubule body. Conception of cap structure raised several fundamental problems concerning microtubule dynamic instability. First, how long is the lag-time between incorporation of heterodimer in microtubule and GTP hydrolysis? A value of two second for this lag-time was measured by stopped-flow enzyme-linked assay (Melki et al., 1996), whereas previous report of Stewart and colleagues indicated the absence of such a gap (Stewart et al., 1990). Structural characterization of cap is another experimental problem. Different studies gave distinct estimation of the GTP-cap size, but many indicated that this cap is a very small structure containing several tubulin layers (Desai *et al.*, 1997). Surprisingly, it was also calculated that only a single layer of GTP-tubulin was necessary and sufficient for stabilization of microtubule end (Caplow and Shanks, 1996). It is worthy to note that in living cells dynamic instability is most probably related only to “plus” ends of microtubules, because “minus” ends are often protected against depolymerization by association with capping proteins.

A.3.4. Factors of microtubule dynamics *in vivo*

Amazingly cellular microtubules exhibit a ten time higher polymerization rate, for given tubulin concentration than microtubules *in vitro*. Paradoxically, the catastrophe frequency for cellular microtubules is also higher. Thus, cellular microtubules are more dynamic structures, than microtubules formed *in vitro* (Kinoshita et al., 2001). One of the most exciting examples of modulating microtubule dynamic *in vivo* are disrupting of the interphase microtubule network during interphase-mitosis transition. During interphase microtubules are more stable with a half-life time of about 10 minutes, whereas in mitosis the half-life of single microtubules is about 30 seconds (Jordan and Wilson, 1998). Another example of such variations is the rapid change in the cellular microtubule network in Xenopus oocytes in response to fertilization (Gard and Kirschner, 1987b).

The difference between microtubules made *in vitro* with pure tubulin and microtubules *in vivo* can be understood if we consider the existence of multiple

cellular factors regulating microtubule dynamics (Gard and Kirschner, 1987a;Walczak et al., 1996;Curmi et al., 1999;Kinoshita et al., 2002).

A.3.4.1. Small drugs modulating microtubule dynamics

Numerous natural microtubule-targeting drugs were found in plants and animals that use these toxic molecules as passive defense. Nowadays some of these drugs serve in medicine as effective anti-cancer agents that arrest the cell cycle and inhibit proliferation of cells. As mentioned above, microtubules become more dynamic when cells enter mitosis. The fundamental reasons of this phenomenon are still obscure; however this higher dynamics is necessary for the formation of the mitotic spindle and proper chromosomal segregation (Kline-Smith and Walczak, 2004). The failure of mitotic spindle formation or its stabilization at the stage of chromosome segregation results in mitotic arrest. The most important drugs that act on spindle formation are colchicine, vinca alkaloids and paclitaxel (taxol).

Colchicine is a highly toxic natural product that was originally extracted from autumn crocus. It is used in clinics since first century of Common Era for the treatment of gout and also used to generate plant polyploidy for agricultural purposes. Colchicine is not used alone for cancer treatment because of its significant toxicity. Colchicine binds to specific site on β-tubulin close to interface between monomers (Figure 9, A).

Binding of colchicine stabilizes a curved conformation of the tubulin heterodimer, which impedes longitudinal contacts in protofilament and leads to microtubule depolymerization (Figure 9, B) (Ravelli *et al.*, 2004). Podophyllotoxin is a non-alkaloid analog of colchicine used in medicine for treatment of papillomas derived from root of mandragora. Podophyllotoxin competes with colchicine binding to the same site on β subunit. In contrast to these drugs, vinca alkaloids (e.g. vinblastine and vincristine) bind to a region of β-tubulin at the longitudinal surface between two dimers (Gigant et al., 2005). Both types of drugs induce at submicromolar concentration a curvature of protofilament and opened conformation of intra-dimer interfaces. Interestingly, at slightly higher concentrations vinblastine behaves as an interfacial drug with binding site on the longitudinal interface on both α and β tubulin subunits (Figure 9, C). It thus favors tubulin-tubulin interaction and induces the formation of spiral structures that

can self-organize in paracrystals inside the cell (Takanari et al., 1994;Nogales et al., 1995a;Gigant *et al.*, 2005). Vinca alkaloids that were isolated from periwinkle plant are nowadays used as effective drugs to treat childhood leukemia (Jordan and Wilson, 2004). However, they have some side effects like neurotoxicity and myelosuppression. These effects are related with the interference of the drugs with neuronal transport and the normal proliferation of bone-marrow cells.

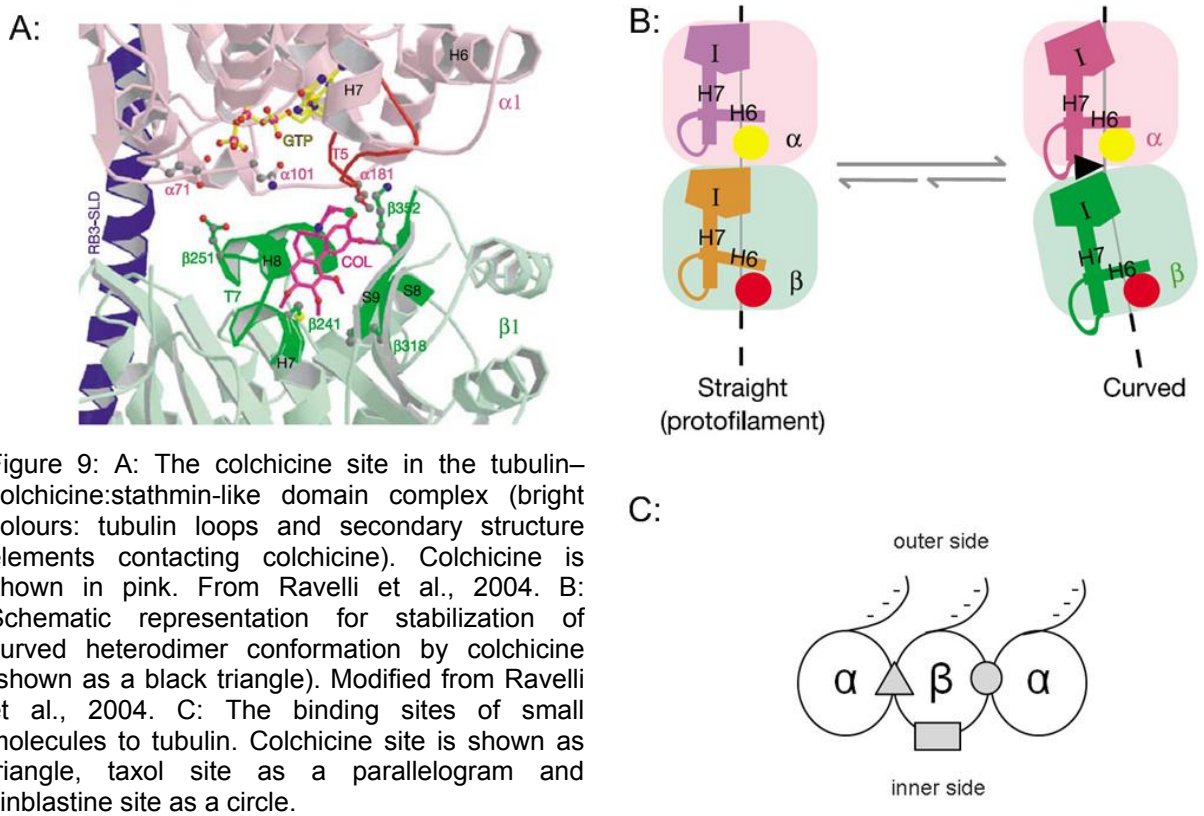


Figure 9: A: The colchicine site in the tubulin–colchicine:stathmin-like domain complex (bright colours: tubulin loops and secondary structure elements contacting colchicine). Colchicine is shown in pink. From Ravelli et al., 2004. B: Schematic representation for stabilization of curved heterodimer conformation by colchicine (shown as a black triangle). Modified from Ravelli et al., 2004. C: The binding sites of small molecules to tubulin. Colchicine site is shown as triangle, taxol site as a parallelogram and vinblastine site as a circle.

Vinca alkaloids depolymerize HeLa cells microtubules at concentrations above ten nanomolar. At nanomolar concentrations vinca alcaloids doesn't produce any significant effect on microtubule mass, but still block cellular proliferation and induce apoptosis. These effects are related to an alteration of microtubule dynamics (Jordan et al., 1992;Honore et al., 2005). Low concentrations of drugs are highly clinically relevant because of reduced side effects. The inhibition of cell proliferation by low doses was attributed to an alteration of the microtubule dynamic instability. It was proposed that as low as a few molecules of drug bound to microtubule end are sufficient to stabilize microtubule. Indeed it was measured that these doses reduce both rates of catastrophes and rescues and increase the time when microtubules are paused (Toso et al., 1993;Dhamodharan et al., 1995). Paclitaxel (taxol) is the most

important member of another microtubule-targeting drug family. It was isolated from the bark of yellow tree in 1969 during American anti-cancer screening program (Cragg, 1998). It was discovered years after that it clearly stimulated microtubule polymerization (Schiff et al., 1979). At a concentration of 80 nM taxol increases the microtubule mass in HeLa cells by a factor two and acts as a strong microtubule stabilizer. However, nanomolar concentration of taxol doesn't produce any detectable effect on microtubule mass, but interfere with mitotic spindle formation (Jordan et al., 1993). Taxol blocks mitosis of HeLa cells even at 8 nM concentration, when concentration of taxol is several hundred times less, than that of cellular tubulin. It is accepted that taxol binds to β-tubulin on the inner surface of microtubule and probably stabilize lateral contacts between protofilaments (Nogales et al., 1995b). The clinical use of taxol is relatively wide, including treatment of ovarian, breast and lung cancers.

A.3.4.2. Regulation of microtubule dynamics by protein partners

Numerous cellular proteins regulate microtubule dynamics *in vivo*. These regulators were artificially separated into several functional groups: classical microtubule-associated proteins (MAPs), sequestering/catastrophe proteins (stathmin family), severing proteins (katanin family) and motor proteins (dynein and kinesin families).

a) Microtubule associated proteins (MAPs)

Classical MAPs were discovered and described, in the middle of 70-th by different independent research groups, as proteins that co-purified with brain tubulin through multiple cycle of polymerization (Murphy and Borisy, 1975;Witman et al., 1976;Sloboda et al., 1976). These proteins stimulate microtubule assembly and significantly decrease the critical concentration required for polymerization. They also, as taxol, stabilize microtubules against disassembly and lower microtubule dynamics (Drechsel et al., 1992;Panda et al., 1995a). The best characterized classical MAPs are MAP1, MAP2, tau protein and MAP-4. MAP1A and MAP1B are high molecular weight proteins expressed in neuronal cells (Halpain and Dehmelt, 2006). MAP1B is predominantly expressed in embryonic neurons and gradually

disappears during ontogenesis, whereas MAP1A is expressed in adult neuronal cells. Both of these proteins are synthesized in cells as flexible unstructured polypeptides with molecular mass of about 350 kDa that are highly accessible for cellular proteases. Consequently, these large proteins undergo proteolytic cleavage that generates light chains of MAPs with molecular masses of about 30 kDa. The most prominent function of MAP-1 proteins is to stabilize microtubule against disassembly and support intracellular transport. The knockout of MAP-1 proteins results in abnormal neural development and severe phenotype (Teng et al., 2001). Another family of microtubule associated proteins named MAP2 family includes MAP2, tau and MAP4. Tau and MAP2 are expressed in neurons, whereas MAP4 was found in non-neuronal cells. All proteins of the MAP2 family lack any tertiary structure and are heat-stable which is typical for intrinsically unstructured proteins. The members of the MAP2 family all contain the N-terminal “projection” domain followed by several repetitions of microtubule-binding domain (Amos and Schlieper, 2005). The microtubule-binding domain decreases microtubule dynamics and increase their rigidity (Panda et al., 1995b;Drewes et al., 1998). Structurally microtubule binding domains consist of multiple repetition of the KXGS motif that binds to the inner side of microtubule near the taxol-binding site on β-tubulin (Kar et al., 2003). The projection and microtubule binding domains are connected by a proline-rich region that could thread through the holes between protofilaments and thus link parts of the proteins that are exposed outside and inside microtubule. Electrostatic charges are not distributed equally along MAPs: the N-terminal domain is negatively charged, whereas repeats and proline-rich regions are enriched in basic aminoacids. The proline-rich extended region bridges several tubulin dimers within protofilament and together with repeated region increases microtubule stability, whereas projection domain repels microtubules and provide spacing (Mandelkow and Mandelkow, 1995). Binding of MAP2 proteins to microtubules can be regulated via phosphorylation by protein kinase A and microtubule affinity regulating kinase (MARK) (Sloboda et al., 1975;Drewes et al., 1997). These two kinases phosphorylate the KXGS motif and diminish MAPs affinity to microtubules. Interestingly MAP2 is also able to interact via its microtubule-binding repeat with another negatively charged cellular polymer – filamentous actin (Dehmelt and Halpain, 2004). These results strongly suggest that a significant part of the interaction between MAPs and microtubules is provided by electrostatic attraction.

b) Proteins that destabilize microtubules

Several of the well-known microtubule destabilizing proteins belong to the stathmin family. Stathmin was first identified as a relay phosphoprotein in multiple signal transduction (see (Sobel, 1991) for a review). It seems that stathmin and the other homological proteins (RB3, SCG10 and SCLIP) are intrinsically unstructured. All proteins of stathmin family contain multiple phosphorylation sites that modulate their activity (four sites in the case of stathmin). Later on, it was discovered that the presence of stathmin destabilize microtubules, an effect first attributed to an increase of the level of catastrophes (Belmont and Mitchison, 1996). It was soon demonstrated that this effect also results from the sequestration of tubulin in a tertiary complex made of two tubulin heterodimers per stathmin molecule (T_2S complex) (Curmi et al., 1997;Jourdain et al., 1997). The structural investigation of tubulin in the sequestration complex led to the first crystal structure of tubulin (Gigant *et al.*, 2000). Thought the resolution of this structure was moderate (4 Å) it revealed that the stathmin molecule stabilizes a curved conformation of the pair of tubulin heterodimer. More recently the same group obtained better structures which solved nearly all stathmin molecule and also provided location of colchicine and vinblastine (Ravelli *et al.*, 2004;Gigant *et al.*, 2005).

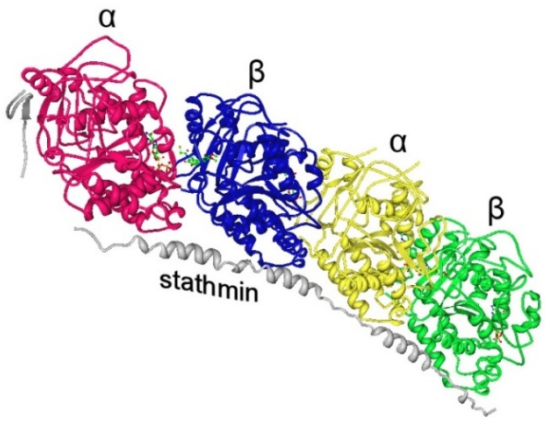


Figure 10: Structure of stathmine:tubulin complex (PDB ID: 1SA0). Picture was made with Protein Workshop software.

It was observed that the amino-terminal end of stathmin binds to the α-tubulin subunit at the longitudinal interdimer interface. Both dimer curvature and steric hindrance of α-tubulin subunit by the N-terminal part of stathmin prevent incorporation of tubulin dimer inside the microtubule lattice. Notably, stathmin is unable to destabilize microtubule preformed in presence of GMPCPP that also argues in favor to the tubulin-sequestering model of stathmin action (Walczak, 2000). However, recent data show that stathmin strongly increases the catastrophe frequency at the minus ends due to direct interaction with pre-polymerized microtubules (Manna et al., 2006). Altogether these data propose co-existence of two different mechanisms for regulation of microtubule dynamics by stathmin. Another protein, katanin, also leads

to microtubule depolymerization via a different molecular mechanism. Katanin disrupts contacts between subunits in already preformed microtubules and provokes their depolymerization (Quarmby, 2000). Electronic microscopy and atomic force microscopy studies revealed that katanin forms hexamers on the surface of microtubules in defect places were some tubulin dimers are missing (Amos *et al.*, 2005). The same mechanism of action has been proposed for spastin, a protein mutated in human spastic paraplegia (Hazan et al., 1999;Salinas et al., 2007). Some proteins related to kinesin motor are also involved in the regulation of microtubule dynamics. Contrary to classical kinesin (kinesin-1) that moves cellular cargoes towards plus-ends of microtubules, proteins from the Kinesin-13 subfamily use hydrolysis of ATP to depolymerize microtubule ends. One of the well-known examples of these proteins is XKCM1, a kinesin identified in Xenopus eggs. Inhibition of XKCM1 function leads to increase in microtubule length and disruption of mitotic spindle assembly (Walczak et al., 1996). This protein is able to induce curvature of protofilaments and depolymerize microtubules preformed even in presence of GMPCPP (Walczak, 2000).

A.3.5. Transport along microtubules

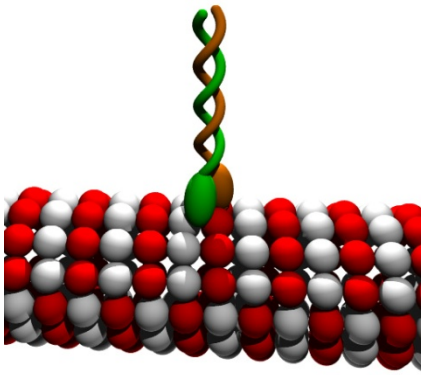


Figure 11: Molecule of kinesine bound to microtubule.

The microtubule network serves for transport of organelles, protein complexes and secretory vesicles inside the cell. Transport of cargoes along microtubules is performed by two types of molecular motors: kinesin and dynein. Kinesin is a dimeric protein consisting of two light and two heavy chains joined together. Structure of kinesin resembles two coiled coil snakes (Figure 11). The head domain is the molecular motor part that possesses both the ATP-ase and microtubule binding activities. The head domain is connected to the tail domain via a coiled-coil “stalk” region. The tail domain attaches different kind of cargoes, whereas the “stalk” domain serves as lever for motor domain (Rice et al., 1999). Kinesin light chains anchor various types of cargoes due to the interaction with numerous adaptor proteins. Moving of kinesin along microtubule is connected with ATP hydrolysis and subsequent conformational change of motor domain together with the “stalk”. The high efficiency of kinesin

results from its ability to convert about 50% of the energy released from ATP hydrolysis into mechanical work (Kikkawa, 2008). Nearly all members of kinesin family carry cargoes to the “plus” ends of microtubules which are oriented toward cell periphery. The other important motor protein is dynein which moves cargoes toward the “minus” end of microtubules. This protein with a molecular weight around 1 MDa consists of numerous subunits and is ten time bigger than kinesin (Mallik and Gross, 2004). Dynein complex possesses two heads linked with each other while cargo interacts with stem region. Both heads contain six AAA domains and some of them are able to binds and hydrolyze ATP. Dynein moves along microtubule surface toward nucleus via repeated cycles of detachment and reattachment of heads, provoked by ATP hydrolysis. It is noteworthy that in cells, dynein movement requires the presence of highly conserved dynactin complex, which consists of several subunits (King and Schroer, 2000).

A.4. PROTEINS THAT MAKE A LINK BETWEEN TRANSLATION AND MICROTUBULE CYTOSKELETON

In addition to their role in the translation of mRNA, several translational factors interact with tubulin and microtubules. One of the most well known examples is eukaryotic elongation factor 1α (EF1a). This protein is a prominent and abundant component of eukaryotic cells playing multiple intracellular roles (Toueille et al., 2007). Beyond its function in polypeptide elongation, EF1a interacts with microtubule cytoskeleton. It has been shown that EIF1a stimulates polymerization of microtubules and induces their bundling in vitro (Durso and Cyr, 1994). In plants cells, EIF1a is partly associated with microtubules and probably acts as a microtubule associated protein regulated by calcium signal transduction pathway. Moreover, in extracts of sea urchin eggs, EIF1a is a major non-tubulin component associated with mitotic spindle and centrosomes. Anti-EIF1a antibody injected in prophase cells totally blocked the formation of the mitotic spindle and cell division (Kuriyama et al., 1990). Interestingly, monoclonal antibody against isolated mitotic spindles recognized EIF1a as the target antigen. On the basis of these observations it was proposed that EIF1a forms microtubule organizing centers inside cells and acts as agent of microtubule nucleation. Most probably, during mitosis it also stabilize mitotic spindle and act as a

classical MAP (Ohta et al., 1990). However, other experiments revealed that EIF1a exhibits microtubule destabilizing activity (Shiina et al., 1994). Indeed it was observed that both native and bacterially expressed proteins effectively disrupted microtubule *in vitro* and after injection in living cells. Together, these results support the idea that EIF1a is implicated in regulation of microtubule dynamics by different means. Complementary data were obtained by isolation of soluble tubulin-containing complexes from CHO and HeLa cells arrested at late prophase with nocodazole (Marchesi and Ngo, 1993). Treatment of cells with nocodazole leads to the accumulation of macromolecular tubulin-containing complexes that can be readily purified by two-step high speed centrifugation procedure from cytoplasmic extracts. These macromolecular complexes were composed with α/β and γ tubulin together with EIF1a. In addition to these components they also contain heat-shock protein 70 or G-actin dependent on the presence of ATP in the isolation buffer. It has been proposed that these complexes represent centrosome precursors, which can form microtubule-organizing center later in mitosis.

Another translation factor involved in cytoskeleton function is eukaryotic initiation factor 4F from wheat germ (Bokros et al., 1995). This factor is composed of two subunits p28 and p86 that serve for different functions. The small subunit, p28, recognizes 7-methylguanine cap structure located at the 5' end of mRNA, whereas the large subunit p86 is responsible for the interaction with other translational factors, such as eIF4A, eIF4B and PABP. Surprisingly, the large subunit of plant eIF-(iso)4F shares structural similarities with the microtubule binding region of kinesin motor protein. However, contrary to kinesin, the p86 subunit of eIF4F doesn't have any ATP binding site and unlike kinesin, binding of eIF-(iso)4F to microtubules is not dependent on the presence of ATP. Interaction of eIF-(iso)4F with microtubules is mainly due to electrostatic interactions, because even slight increase of ionic strength completely removes this factor from microtubules. Functionally, the binding of eIF-(iso)4F to microtubules *in vitro* results in the formation of huge bundles even at a molar ratio of one eIF-(iso)4F molecule per ten tubulin dimer. This molar ratio is quite close to physiological concentration and it is proposes that this factor may be involved in microtubule bundling *in vivo*. Interestingly, p86 increases end-to-end annealing of preformed microtubules under *in vitro* conditions (Hugdahl et al., 1995). The same properties were described for classical microtubule associated proteins

like MAP2 and tau. The annealing of microtubules correlates with a rapid increase in microtubule length and reduction in microtubule number within short period of time. Most probably, the annealing effect of MAPs and p86 correlates with microtubule bundling, because bundled microtubules are well aligned. Finally, immunofluorescence analyses revealed that p86 is diffusely distributed into the cytoplasm, but also forms particles and patches near cortex of maize root cells. These protein clusters are probably attached to membranes and co-localize with microtubule bundles. These results also support the idea that eIF-(iso)4F is a novel microtubule-associated proteins that may link translation apparatus with cytoskeleton.

Interestingly, the life cycle of human immunodeficiency virus (HIV) could be also coupled to cytoskeleton. The RNA of HIV contains a specific stem-loop sequence, named trans-acting responsive element (TAR) that enhances transcriptional initiation, elongation and translation of HIV RNA. At the same time, TAR activates PKR kinase that phosphorylates eIF2 and shuts off translation of host cellular genes, whereas translation of HIV RNA remains unaltered (Schneider and Mohr, 2003). TAR sequence is recognized by viral Tat protein that stimulates viral translation and overcomes the inhibitory effect of RNA leader (Sengupta et al., 1990). It has been shown recently that Tat protein increases microtubule assembly and interacts directly with them (de Mareuil et al., 2005). These data could indicate that HIV infection provokes simultaneous reorganization of cytoskeleton and translational apparatus in order to overcome cellular defending mechanisms and also for effective production of mature virus particles.

A striking example of connection between RNA and the cytoskeleton is provided by studies of transport of viral RNP complexes in plants (Jorgensen et al., 1998). Movement of virus RNA between plant cells does not require coat protein and it is more likely that virus spreads along organism in the form of viral RNP. Plant viruses encode specific movement proteins that pack viral RNA and enable viruses to exploit cellular cytoskeleton for invasion and spreading. One of the most studied examples of such proteins is tobacco mosaic virus movement protein (TMV-MP) (Heinlein et al., 1998). The expression of TMV-MP is mandatory for spreading of infection and even single mutation in the region that share similarity with γ-tubulin (see below), can abolish intercellular transport of viral RNA. Mutant viruses were able to spread between cells at a temperature of 22°C, whereas spreading was completely

abolished at 32°C. In addition, TMV-MP was found to be associated with microtubule cytoskeleton and the associations of mutant protein forms were disrupted at elevated temperatures. These results clearly indicated that complexes of TMV-MP with viral RNA move along microtubules during infection. Surprisingly, microtubules from infected cells are extremely stable against treatment with salts and cold-induced depolymerization. This property is very unusual and remarkable since even classical MAPs can be readily released by addition of salts and are not able to stabilize microtubules against cold disassembly, except the case of STOP protein which action on microtubules is still under discussion (Guillaud et al., 1998). The huge effect of TMV-MP on microtubule stability indicates that this protein is tightly associated with the microtubule lattice, in contrast to classical MAPs. Sequence analysis revealed that TMV-MP does not share any structural homology with MAPs but share homology with γ-tubulin (Boyko et al., 2000). This finding raised the idea that TMV-MP could incorporate into the microtubule lattice and stabilize lateral contacts between protofilaments. Another possible explanation is that TMV-MP may form cap structure at the ends of microtubules and stabilize them. Interestingly, it was observed that mammalian cells transfected with TMV-MP demonstrated an absence of γ-tubulin in the centrosome and the presence of abnormal microtubule arrays that suggested functional similarity between TMV-MP and γ-tubulin. Altogether, these studies proposed a dual role of TMV-MP in transport of virus particles and microtubule assembly. Most probably, viral RNPs serve as microtubule nucleation sites and use microtubule polymerization forces for intra- and intercellular spreading of infection.

B. AIMS OF THE STUDIES PERFORMED FOR MY PH.D.

As mentioned above, transcription, mRNA processing, localization and translation are coupled together via multiple protein-protein interactions that occur both in the cytoplasm and nucleus. This additional layer of complexity in the regulation of gene expression helps cell for adaptation to continuously changing environmental conditions. The participation of YB-1 in rapid and complex processes of cell response to stress indicates its involvement in multiple protein-protein interaction and raises the question about protein partners of YB-1 within cell. YB-1 interactions with DNA and RNA are profoundly described, whereas much less is known about involvement of YB-1 in protein-protein interactions. To date, only few protein partners that interplay with YB-1 were identified, including actin, DEAD-box helicases, p53, tumor transcription factor TLS and hnRNP K.

Chronology of my work is as follows. The first part was totally performed in the laboratory of Prof. Lev Ovchinnikov at the Institute of Protein Research where I worked since 2000. In this lab, I characterized RNP complexes by biochemical methods and atomic force microscopy. At the same time I participated to the study of mechanisms of YB-1-induced translational inhibition. Altogether results of my work in Russia unraveled a link between translational status of mRNPs and their structure.

My work on the interaction of YB-1 with tubulin started in 2006. It was performed at the Institute of Protein Research and INSERM U829 under the co-supervision of Dr. Patrick Curmi and Prof. Lev Ovchinnikov. I identified novel protein partners of YB-1 in different extracts from mammalian tissues and characterized them for better understanding of fundamental cellular processes. This part includes the identification of tubulin as a novel molecular partner of YB-1 and reveals the influence of YB-1 on microtubule assembly.

Then I described the interaction of mRNP with microtubules promoted by the major core mRNP proteins YB-1 and PABP. This work performed at INSERM U829 provides clues for a better understanding of molecular mechanisms of mRNPs transport and localization into cell and proposes a novel link between microtubule cytoskeleton and translation apparatus.

Finally, at INSERM U829, I participated to the development of an original model of microtubule assembly. This model takes into the account the possibility that facilitated diffusion of tubulin along microtubules plays a role in the processes of microtubule nucleation and elongation. The model was evaluated experimentally. Together, it points out the critical participation of polyamines on microtubule assembly and opens interesting perspectives to develop new therapeutic strategies for cancer treatment.

For the sake of clarity, I will describe first the results regarding YB-1:tubulin interaction then the participation of YB-1 in mRNPs formation and the possibility of mRNPs:microtubule interaction. Finally, I will describe the novel microtubule assembly model taking into the account possibility of facilitated diffusion of tubulin.

C. MATERIALS AND METHODS

The methods used in this work are listed in Table 1. References to published methods and modifications are described in attached articles. New methods are described in the original publications.

Table 1: List of methods used in this study.

Methods	Articles
YB-1 purification	I, II, III, IV
PABP purification	III
Tubulin preparation	I, III
Tubulin S preparation	I
Rabbit tissue extracts preparation	I
Affinity chromatography	I
Microtubule sedimentation assays	I, III
Cross-linking of protein and RNP complexes	I, II
Native agarose gel electrophoresis	I, III
In vitro tubulin polymerization assays	I
Synthesis and purification of RNA	I, II, III, IV
Atomic force microscopy	I, II, III
Transmission electron microscopy	I, II
Electrophoretic mobility shift assay	I, III

D. RESULTS AND DISCUSSION

D.1. IDENTIFICATION AND CHARACTERIZATION OF THE YB-1:TUBULIN INTERACTION

(PUBLICATION I)

In order to identify novel YB-1 partners that could modulate gene expression via protein-protein interaction we decided to use YB-1 affinity chromatography as a tool to obtain YB-1 interacting proteins from different rabbit tissue extracts. Indeed, affinity chromatography has been widely used since long time as a valuable and trustable physical method to select, detect and purify proteins that bind particular target. Different target proteins can be coupled to a matrix under physiologically relevant conditions that preserve their activity and used to catch partners from appropriate extracts. Most of non-specific and weakly bound compounds are removed from column in buffer with low salt concentration, whereas truly specific and strong interacting partners can be eluted only by harsh treatment including high salt or addition of detergents.

Affinity chromatography possesses many advantages compared to other methods for detection of protein-protein interaction. The most important property of this method is simplicity and high reproducibility. The other advantage is elevated sensitivity, indeed, even very weak interaction between target and minor protein component from extract could be detected easily in the range of binding constants of 10^{-5}-10^{-3} M. Finally, the presence of protein competitors within cell extracts helps to eliminate artificial and physiologically irrelevant interactions (Phizicky and Fields, 1995).

Recombinant YB-1 was thus immobilized via amino groups on a CNBr-activated matrix. YB-1 was purified from *E. coli* transformed with pET3-1-YB-1 construct. Purification procedure included ultracentrifugation of E. coli extract, heparin-Sepharose chromatography and gel-filtration on Sephacryl S-200 columns. Purity of YB-1 protein estimated by SDS-PAGE was more than 90 %. The procedure of YB-1 purification is described in more details in article I and elsewhere (Ustinov et al., 1996;Evdokimova et al., 2001). Before coupling, purified YB-1 was dialyzed against carbonate buffer containing high concentration of salt in order to prevent YB-1 adsorption on the surface of dialysis tube. Buffer exchange was done also in order to

remove traces of Tris-HCl buffer that can effectively compete with protein amino groups for reaction with CNBr-Sepharose.

Two one-year old male adult rabbits were used to obtain fresh tissues. Rabbits were killed by decapitation and organs were isolated within 30 min. All organs were washed and homogenized in physiological buffer in motor-driven homogenizer. Homogenates were centrifuged at low speed to pellet cell debris, supernatants were collected and frozen rapidly in liquid nitrogen. The protein concentration in resultant extracts was between 20-50 mg/ml as measured by Bradford assay.

First, we assessed YB-1 distribution in tissue extracts using rat polyclonal anti-YB-1 antibody kindly provided by Dr. A. Sorokin. Rat primarily antibody were chosen to avoid cross-reaction between rabbit antibody presented in tissue extracts and secondary anti-rabbit antibody.

As seen on Figure 12, YB-1 is readily detected in testis, kidney, liver and spleen tissue extracts. This distribution of YB-1 is in agreement with previous report on mouse tissues, indicating that YB-1 is present in adult spleen, testis, liver, kidney and lung (Miwa et al., 2006).

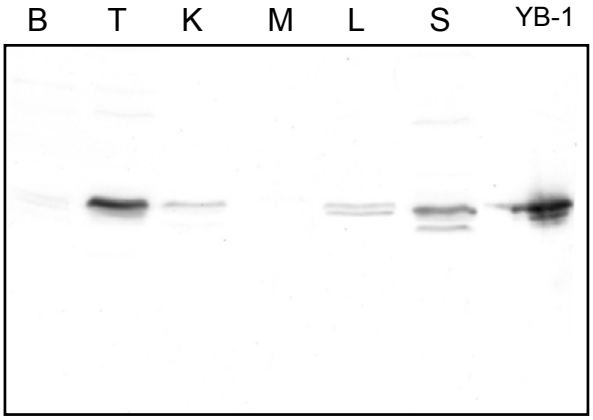


Figure 12: Distribution of YB-1 in rabbit tissue extracts. Rabbit tissue extract proteins were separated on 12% SDS-PAGE, transferred onto a membrane and probed with rat anti-YB-1 antibody followed with secondary antibody. Bands were revealed using the ECL technique. B-brain, T-testis, K-kidney, M-muscle, L-liver, S-spleen.

The clarified tissue extracts were diluted with buffer to obtain a protein concentration of 1 mg/ml and incubated with YB-1-Sepharose. The slurry was then washed with low-salt buffer and bound proteins eluted with high salt buffer. Results of YB-1 affinity purification are displayed on Figure 13. As seen, only a few proteins from rabbit extracts were retained on affinity resin and eluted with high salt. In the same conditions, BSA Sepharose didn't retain any proteins that indicated specific affinity of bound proteins for YB-1. Eluates from YB-1 Sepharose contained two prominent bands with apparent molecular weights of 50 and 45 kDa when brain, liver and kidney extracts were used as source. An additional 90 kDa protein was eluted in the case of muscle extracts. The prominent bands of 90 kDa, 50 kDa and 45 kDa were excised from gels, proteins were eluted and digested with trypsin. Products of digestion were lyophilized, separated by MALDI and resultant peaks were analyzed with the MASCOT software. Result of protein identification by peptide mass fingerprint is presented in Table 2. As seen, the 50 kDa band was identified as a mix of tubulin subunits, the 45 kDa brain protein was identified as actin and the 90 kDa protein from muscle corresponded to glycogen phosphorylase. Relatively high probability scores (more than 65) and coincidence between experimentally determined and calculated molecular masses of proteins suggest that proteins were correctly identified.

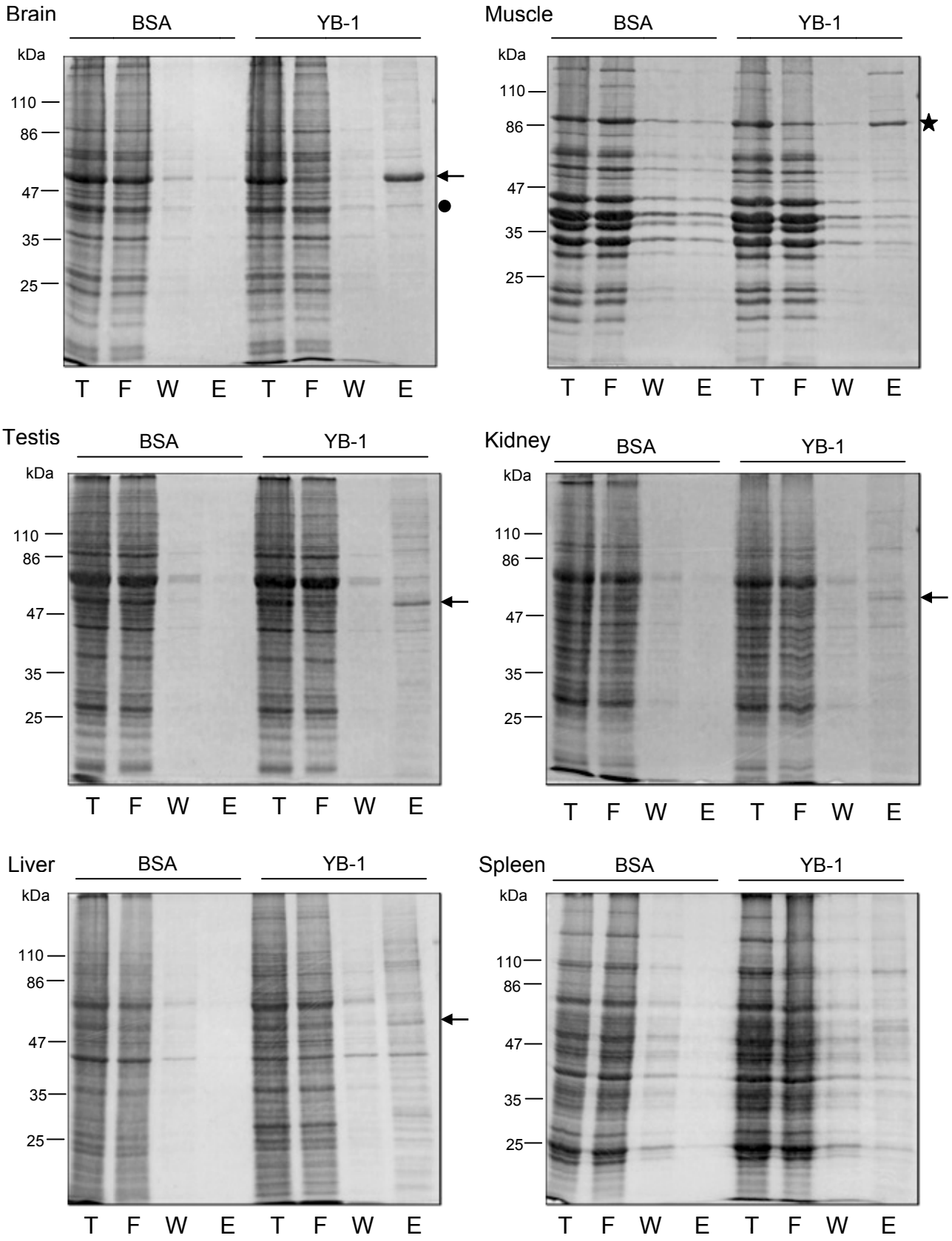


Figure 13: Affinity purification of YB-1- interacting proteins. Tissue extracts (T), flow-throws (F), low-salt wash (W), and high salt eluates (E) are presented. Positions of molecular weight markers are indicated at the left, arrows indicate position of the 50 kDa band, dot and asterisk indicate positions of the 45 and 90 kDa bands respectively.

Eluate	M_r theoretical (experimental), kDa	Accession no.	Score	Name of protein
brain	51 (50)	AAA91576	77	alpha-tubulin
brain	41.7 (45)	AAH12854	127	actin
testis	50 (50)	AAC39578+EAW88369	275	alpha-tubulin+beta-tubulin
kidney	50 (50)	AAC39578+EAW88369	289	alpha-tubulin+beta-tubulin
liver	50 (50)	CAA25855+NP_006079	157	alpha-tubulin+beta-tubulin
muscle	94 (90)	NP_002854	119	glycogen phosphorylase

Table 2: YB-1 interacting proteins identified by MALDI-MS.

In addition to tubulin, eluates from YB-1 Sepharose contained significant amounts of other proteins, as shown on Figure 13. To confirm that YB-1 interacts with tubulin independently on the presence of other proteins, we purified tubulin from sheep brain extract using subsequent cycles of tubulin polymerization/depolymerization in buffer containing high concentration of glutamate (Castoldi and Popov, 2003). This procedure allows removal of all microtubule associated proteins from tubulin preparation without phosphocellulose chromatography step. Purified tubulin was applied onto YB-1-Sepharose column and column was washed with increasing concentration of salt. As shown on Figure 14 (upper panel), pure tubulin was bound to the YB-1 column at physiological ionic strength and up to 300 mM, whereas it was eluted at higher salt concentration. This result indicates that YB-1:tubulin interaction decreases upon increasing of ionic strength.

It is known that tubulin can form microtubules upon polymerization *in vitro*. We then tested the possibility that YB-1 may bind to microtubules and the resistance of that association to increasing concentrations of salt. As demonstrated on Figure 14 (middle panel), YB-1 was bound to taxol-stabilized microtubules under physiological and moderate ionic strength conditions, whereas all YB-1 was removed from microtubules at 600 mM. Thus, interaction of YB-1 with tubulin and microtubules is moderately susceptible to an increase of ionic strength. Additional experiment for competition between YB-1 and highly positively charged poly-L-lysine was performed to ultimately test contribution of ionic forces in YB-1:tubulin interaction. As seen on figure 14 (lower panel), all YB-1 is displaced from microtubules by addition of 0.6 mg/ml of poly-L-lysine. This result strongly suggests that the interaction of YB-1 with

tubulin and microtubules is partly electrostatic. It is worthy to note that the interaction of brain microtubule associated proteins with microtubules is also sensitive to increasing concentration of salt. Most of microtubule associated proteins can be eluted from taxol-stabilized microtubules at ionic strength higher than 200 mM (Melki et al., 1991), which is similar to what we observed with YB-1.

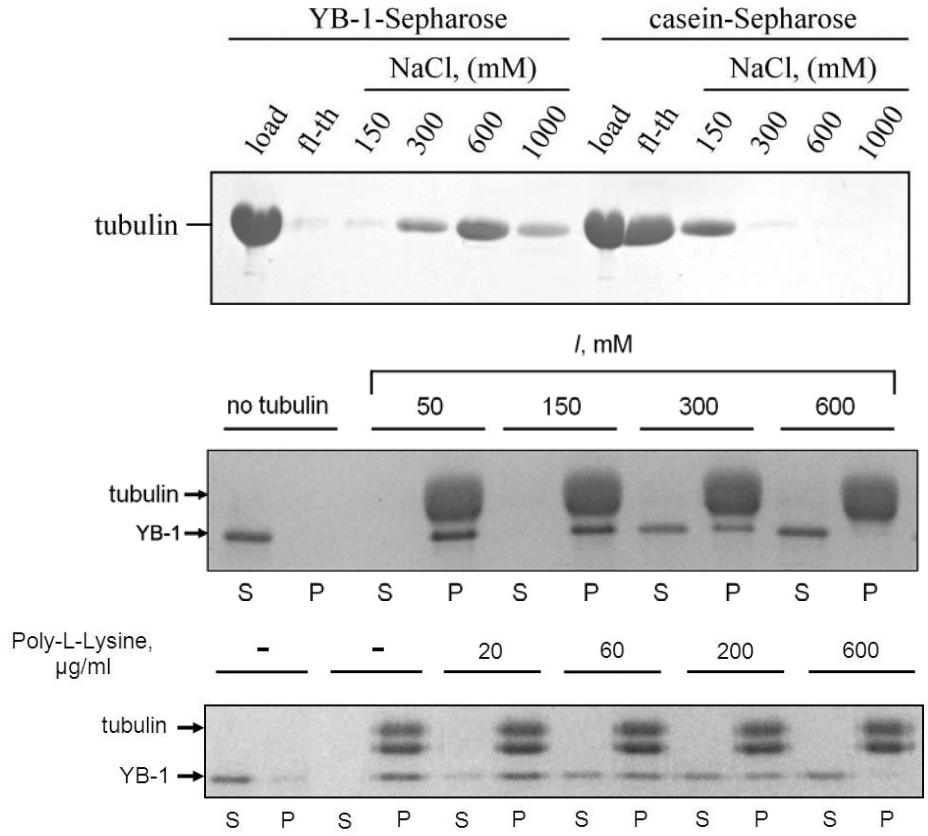


Figure 14: YB-1: tubulin interaction is partly electrostatic.

Upper panel: Tubulin started to elute from YB-1-Sepharose column at 0.3 M NaCl.

Middle panel: YB-1 was totally removed from stabilized microtubules at 0.6 M KCl.

Lower panel: YB-1 can be displaced from microtubules with highly cationic molecules, like poly-L-lysine.

As described in the introduction, the process of tubulin polymerization is regulated by numerous cellular components, including small molecules and tubulin protein partners. The interaction of positively charged molecules with tubulin enhances tubulin polymerization and stabilizes microtubules (Erickson and Voter, 1976a;Mithieux et al., 1984c;Wolff, 1998a). As YB-1 is highly positively charged at physiological pH, we decided to explore effect of YB-1 on microtubule formation. As shown on Figure 15 (left panel), the gradual addition of YB-1 to tubulin assembly mixture strongly decreases the lag-time, increases velocity of tubulin polymerization and plateau value at steady-state. To document the latter effect, soluble tubulin and microtubule pellets were analyzed by microtubule sedimentation assay and SDS-PAGE (Figure 15, right panel). In control conditions when 20 μM tubulin was incubated in absence of YB-1, the major part of tubulin was found in the supernatant in a non-polymerized form. The presence of 5 μM YB-1 significantly increased the amount of polymerized tubulin, compared to control. Higher concentrations of YB-1 didn't produce significant additional effect on microtubule mass (Figure 15, right panel). This indicates that the positive effect of YB-1 on microtubule assembly is saturable.

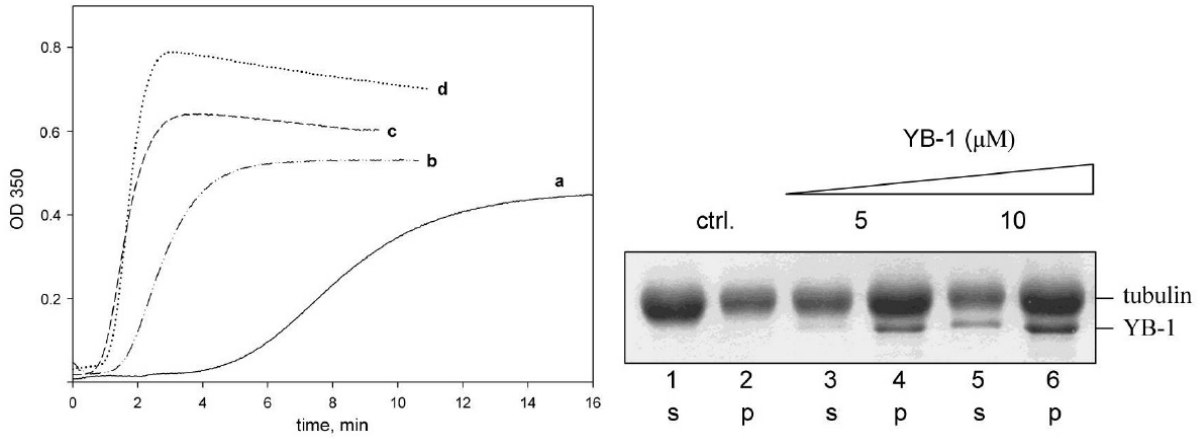


Figure 15: Effect of YB-1 on microtubule assembly in vitro.

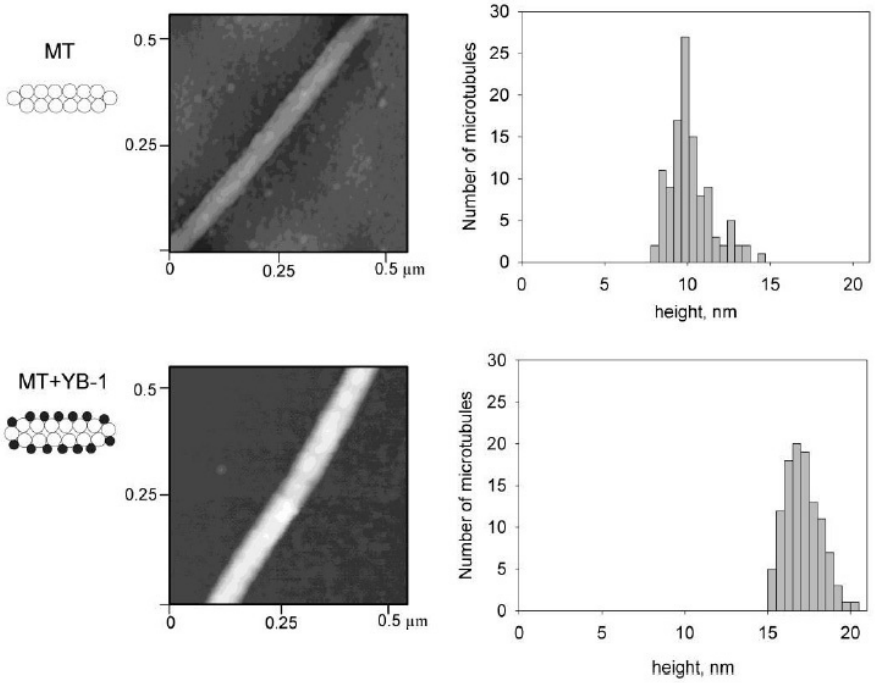
Left panel: Different amounts of YB-1 were added to 20 μM tubulin and polymerization was detected by turbidimetry.

Right panel: Samples were centrifuged at steady-state to pellet microtubules. Supernatants and pellets were analyzed by SDS-PAGE.

It is known that polycations can provoke tubulin polymerization into different aberrant structures and double-walled microtubules (Erickson and Voter, 1976b). To exclude

this possibility, we examined the morphology of YB-1-induced tubulin polymers by AFM and transmission electron microscopy (TEM) imaging. Under control conditions, i.e., without YB-1, microtubules appeared on AFM images as straight rods with an apparent height of 10 nm. This indicates that they were flattened on the mica surface due to the drying procedure (Figure 16(i), top) and the height measurement thus corresponded to two tubulin layers in close contact (see (Vater et al., 1995) and Fig. 16(I), top). Microtubules formed in the presence of YB-1 were significantly higher than the control ones with an average height of about 17 nm. This height could correspond to two layers of YB-1 coating the microtubule outer wall (Figure 16(I), bottom). However, such morphology can also result from microtubules with double walls or with a higher number of protofilaments. To distinguish between these scenarios, we investigated the effect of YB-1 on the ultrastructure of microtubules by TEM performed on thin sections of EPON-embedded microtubules. In control conditions, TEM analyses showed regular microtubules with a diameter of about 25 nm. In the presence of YB-1, the ultrastructure of microtubules remained normal with a single layer of tubulin forming their wall and an outer diameter comparable to control (Figure 16(II), compare D' with C'). This supported further the suggestion that YB-1 coats the outer surface of the microtubule. It is also worth noting that, under the control conditions, microtubules could often be found in close contact with each other (Figure 16(II), C and C'), whereas in the presence of YB-1, microtubules appeared regularly distributed and spaced from each other (Figure 16(II), D and D'). Due to its coating the microtubule surface, YB-1 could induce a change in the rigidity of microtubule or steric hindrance on its outer surface, which may lead to a larger inter-microtubule spacing.

(I)



(II)

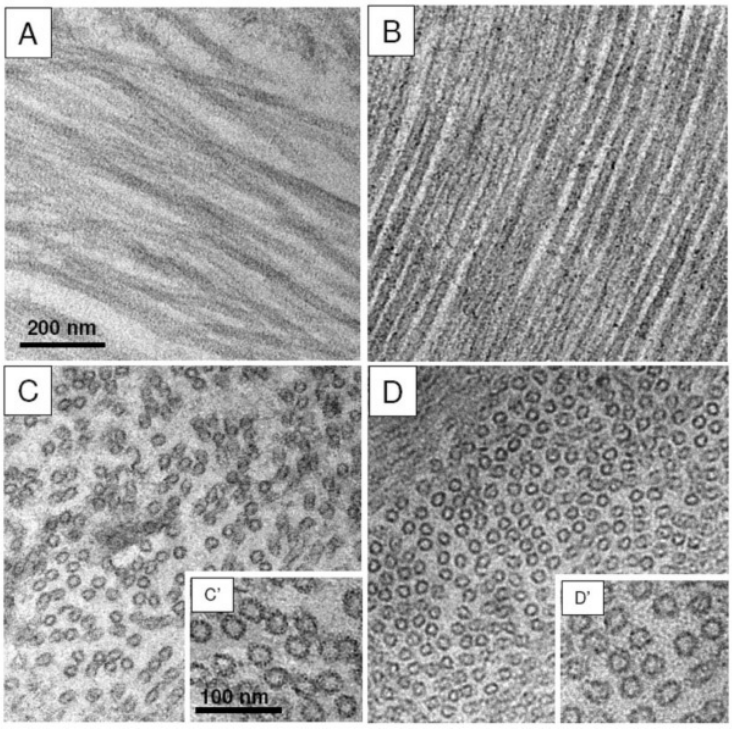


Figure 16: (I): AFM images of normal (top) and YB-1-coated microtubules (bottom). (II): TEM of longitudinal ultrathin sections revealed that YB-1 contributes to the formation of normal microtubules and that, in addition, the microtubules remain regularly spaced from one another (compare B with the control A). Transversal sections of microtubules (C, D) confirmed this observation. B, C, D are at the same magnification as A. D' is at the same magnification as C.

As mentioned above, tubulin assembly requires the formation of relatively small aggregates at the beginning of polymerization that act as nuclei for microtubule growth. Usually crude brain tubulin preparation contains significant amount of 18 S and 30 S oligomeric species, whereas pure tubulin sediments as 6 S particles (Marcum and Borisy, 1978). Recombination of tubulin with microtubule associated proteins leads to the formation of ring-shaped oligomers and increases microtubule assembling capacity (Murphy *et al.*, 1975). These complexes between tubulin and microtubule-associated proteins with an outer diameter of about 30 nm serve as nuclei for microtubule growth at the beginning of polymerization (Scheele and Borisy, 1978;Vallee and Borisy, 1978). The acceleration of microtubule assembly could also involve the formation of oligomeric YB-1:tubulin complexes that act as a nuclei. To characterize YB-1:tubulin complexes we performed biochemical and atomic force microscopy analyses. First, we used 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) to generate zero-length cross-links between YB-1 and tubulin. EDC reacts with a carboxyl at pH of about 6 to form an amine-reactive O-acylisourea intermediate. This relatively unstable intermediate then reacts with N-hydroxysulfosuccinimide hydrochloride (NHS) which results in semi-stable amine-reactive NHS-esters. Subsequent reaction of intermediate with primary aminogroups generates peptide bond and cross-link proteins (Grabarek and Gergely, 1990). After incubation, reactions were quenched by the addition of SDS-PAGE sample buffer and complexes were analyzed by 10 % SDS-PAGE. We found that incubation of isolated YB-1 with EDC/NHS results in the appearance of new bands corresponding notably to protein dimer (Figure 17), which is in agreement with previous data showing that YB-1 possesses ability for self-oligomerization (Izumi et al., 2001;Skabkin *et al.*, 2004). Similarly, cross-linker stabilizes tubulin dimers and little amount of high molecular weight oligomers. Addition of YB-1 to tubulin provokes the gradual disappearance of tubulin dimer and the apparition of a new band with molecular weight of about 150 kDa, containing both tubulin subunits and YB-1 as revealed by immunoblots. Compared with tubulin alone, YB-1:tubulin complexes are also enriched in oligomeric species containing several protein molecules. The result of cross-linking experiment suggests that YB-1 interacts with both α and β tubulin subunits in physiological conditions and promotes tubulin oligomerization, similar to that observed for microtubule associated proteins.

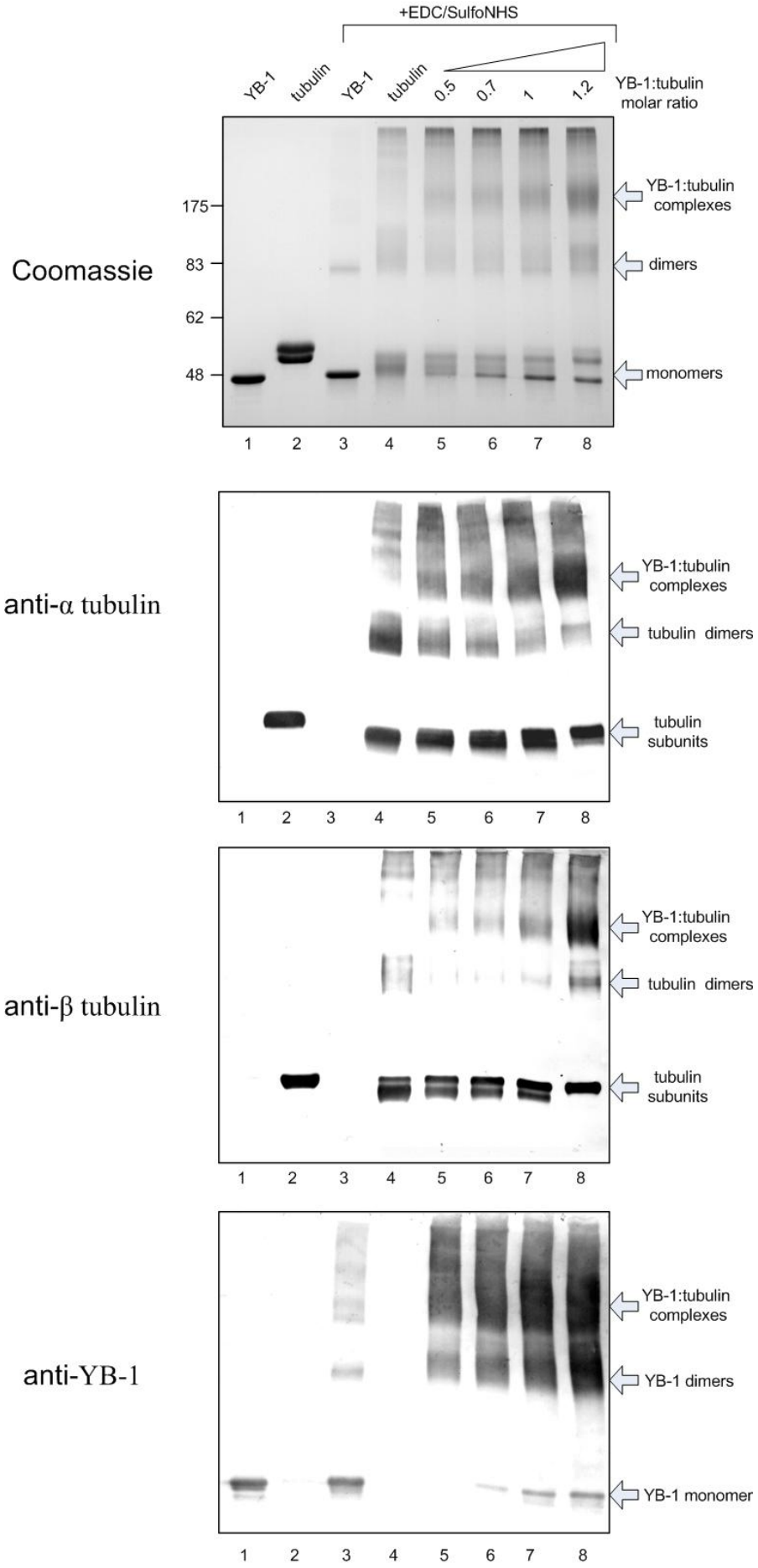


Figure 17: YB-1: tubulin cross-linking.

In order to investigate the morphology of YB-1:tubulin complexes they were imaged by AFM. Complexes were formed by incubation of equimolar amounts of YB-1 and tubulin, fixed with 0.2% glutharaldehyde and adsorbed on mica (Pastre et al., 2003). YB-1 appeared as small and discrete particles on the mica surface with an average height of 0.7 nm (Figure 18, upper panel). This value appeared lower than previously reported by Skabkin (Skabkin *et al.*, 2004) (about 4 nm for monomeric YB-1 in solution of high ionic strength). However, previous AFM experiments were performed using aminopropylsilatran-treated mica (APS-mica) plates as substrates (Shlyakhtenko et al., 1998). Usually this treatment leads to discharging of highly negatively charged mica to promote nucleic acids binding to the surface (Kiselyova et al., 2001). In our conditions, the absence of discharging causes the flattening of YB-1 on untreated mica due to strong electrostatic attraction between positively charged protein and negatively charged surface. In contrast to YB-1, negatively charged tubulin appeared as particles with an average height of 3.7 nm in agreement with previous reports (Vater *et al.*, 1995). In the YB-1:tubulin sample, in contrast to isolated proteins, particles were less homogenous with a size distribution ranging from about 3 to 8 nm (Figure 18, bottom panel). A novel class of particles was clearly distinguishable, centered around 7 nm (Figure 18, lower panel). It was attributed to the YB-1:tubulin complexes probably made of several molecules of both tubulin and YB-1. In addition, we found that YB-1 did not provoke the formation of huge aggregates containing tubulin, in contrast to other highly cationic proteins like histones (Mithieux et al., 1984a). This also indicated the involvement of a few YB-1 and tubulin molecules in formation of these complexes. Most probably, such complexes represent intermediates of tubulin polymerization process and stimulate microtubule assembly.

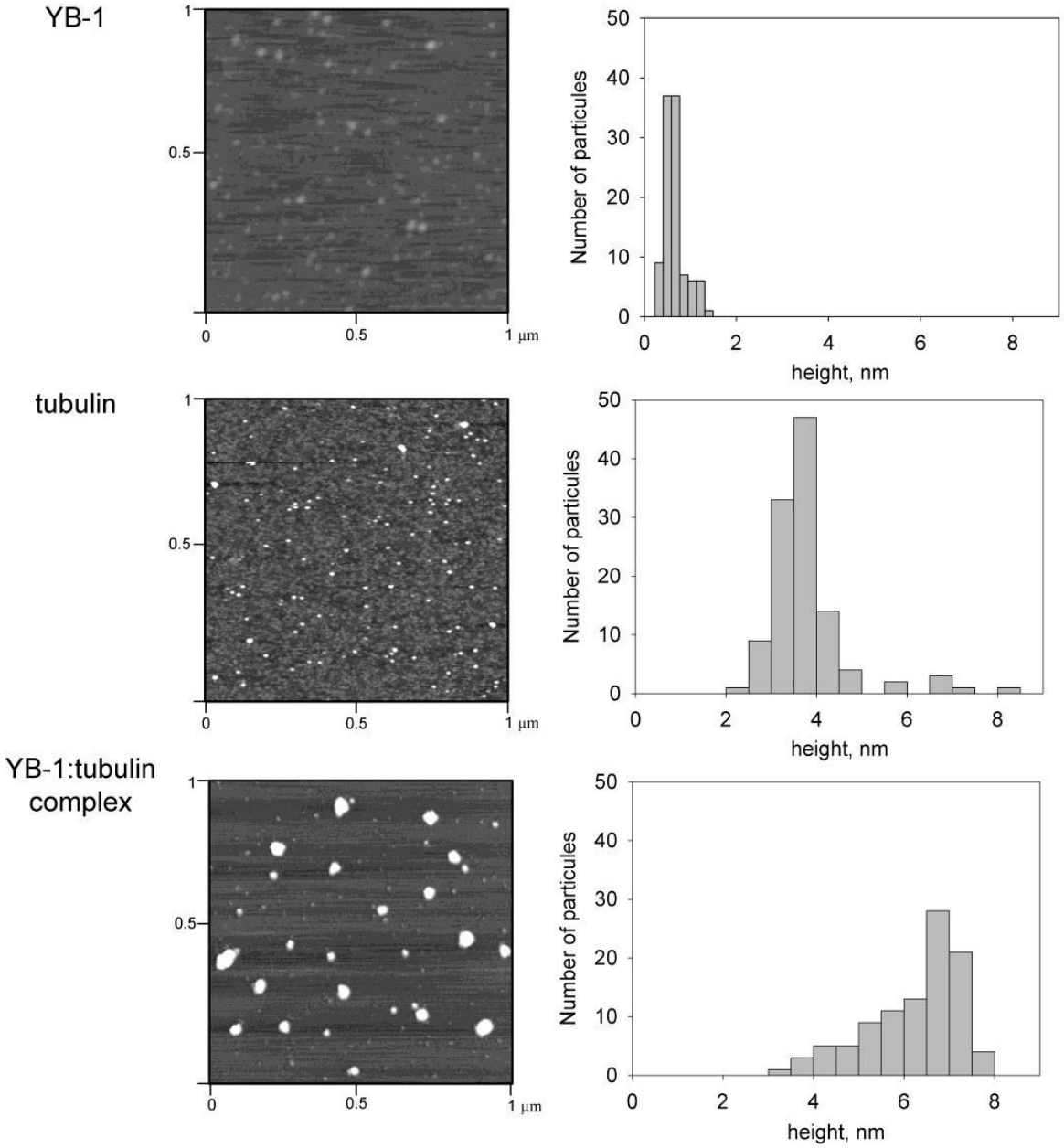


Figure 18: AFM images of YB-1, tubulin and YB-1: tubulin complexes.

Both tubulin subunits are acidic proteins with an isoelectic points about 5.4 (Sackett et al., 1985a). The negative charge of tubulin monomers is unevenly distributed on subunits and concentrated in their C-terminal parts enriched in acidic aminoacids, remarkably glutamic acid (Ponstingl et al., 1981;Krauhs et al., 1981). Thus, at physiological pH C-termini repel each other and impede incorporation of tubulin subunits into polymer. Another notable feature of C-terminal ends is their extended conformation and high susceptibility to chemical modification (Otter et al., 1991;Mejillano and Himes, 1991b;Nogales *et al.*, 1998). Converting of carboxyl into amides leads to a decrease in tubulin negative charge and a consequent reduction of the critical concentration for polymerization (Mejillano and Himes, 1991a). The charge-charge repulsion between subunits can also be relieved by proteolytic cleavage. Limited proteolysis of tubulin with subtilisin removes 2-4 kDa fragments of C-terminal moiety and generates tubulin S which demonstrates a 50-fold lower critical concentration, when compared to uncleaved tubulin (Bhattacharyya et al., 1985;Sackett et al., 1985b). It is worthy to note that limited proteolysis interferes with MAP:tubulin interaction and renders microtubule assembly MAP-independent (Maccioni et al., 1988). YB-1 is highly positively charged at neutral pH, so it was reasonable to propose that YB-1 interacts with tubulin and accelerates microtubule assembly via interaction with C-termini. It was also critical to investigate whether the effects of YB-1 on microtubule assembly could result only from a charge effect or could also be partly due to more specific molecular recognition. For this purpose, we investigated the effect of YB-1 on the assembly of the cleaved tubulin product (tubulin S, Figure 19). Cleavage of both tubulin subunits was assessed by SDS-PAGE and native agarose gel electrophoresis. Interestingly, the polymerization assays revealed that YB-1 was still able to promote the assembly of tubulin S (Figure 19 B). A strong stimulation of microtubule assembly was observed when YB-1 was

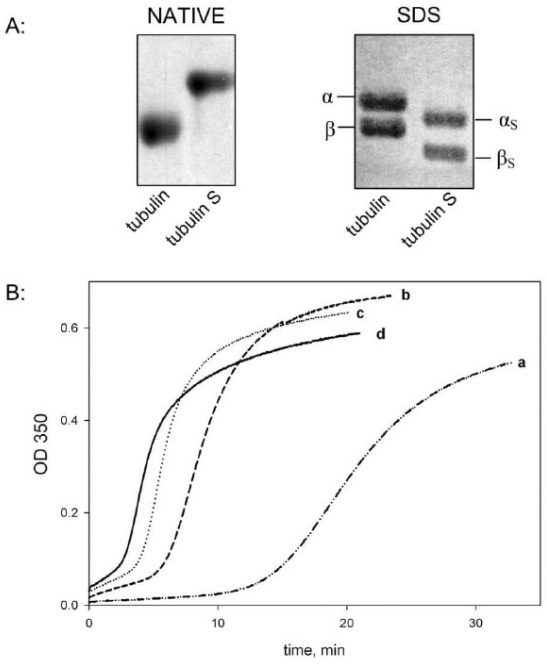


Figure 19: YB-1 favors tubulin S assembly.

added at a total YB-1-tubulin S molar ratio as low as 0.13. At this ratio, YB-1 significantly decreased the lag-time and increased the velocity of the polymerization (Figure 19 B, curve b). Higher concentrations of YB-1 further reduced the lag-time and increased the rate of tubulin S assembly but did not produce any significant additional effect on microtubule mass at steady-state (Figure 19 B, curves c and d). Thus, in contrast with classical microtubule associated proteins, YB-1 accelerates assembly of subtilisin-cleaved tubulin. Together, these data suggest that the promotion of microtubule assembly by YB-1 involves interaction between specific sites of the partners, although non-specific electrostatic interactions may also play an important role. Most probably, YB-1 and MAPs interact with slightly different parts of the tubulin molecule and microtubules. This latter statement was also supported by examining the effect of YB-1 on the polymerization of crude tubulin preparations. It is well documented that the presence of MAPs strongly influences the kinetics of microtubule assembly. MAPs favor the nucleation of microtubules, increase the rate of assembly, extent of polymerization and stabilize microtubules against disassembly (Sloboda *et al.*, 1976;Bulinski and Borisy, 1980;Desai *et al.*, 1997). To investigate whether YB-1 may also influence tubulin polymerization in conditions closer to the cellular ones, we performed a series of experiments with MAPs-tubulin. This preparation contained approximately 15% of MAPs (w/w) as estimated by Coomassie staining of proteins separated on SDS-PAGE. The addition of YB-1 to MAPs-tubulin at a total YB-1:tubulin ratio of 0.3 decreased the lag-time similarly to what we observed for pure tubulin, increased the rate of polymerization and slightly increased the final microtubule mass as estimated from the steady state plateau value (Figure 20). Higher amounts of YB-1 further reduced the lag-time of polymerization but did not change significantly the mass of polymerized tubulin. These data indicate that the presence of MAPs does not abrogate the positive effect of YB-1 on the overall kinetics of microtubule assembly. Together MAP-tubulin and tubulin S polymerization

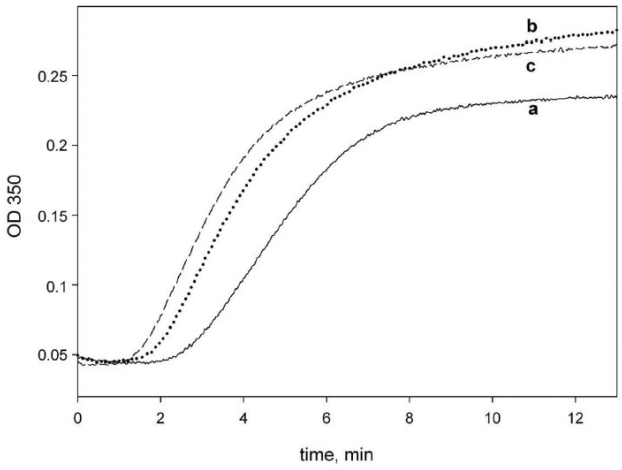


Figure 20: YB-1 favors MAPs-tubulin assembly.

assays demonstrate that YB-1:tubulin interaction possesses properties different from a trivial electrostatic attraction. In this context we believe that YB-1 could be the prototype of a novel class of tubulin-interacting proteins.

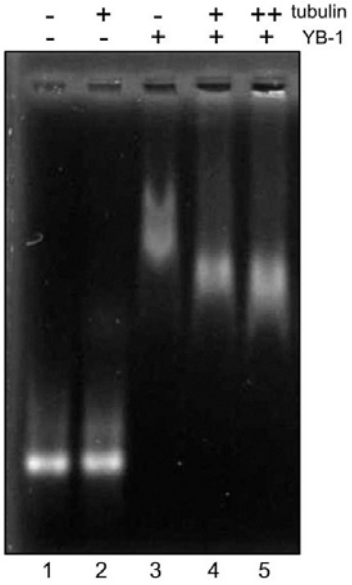


Figure 21: Tubulin modifies the structure of YB-1-RNA complexes.

Finally, we decided to explore whether tubulin can interfere with the formation of RNPs and induce some modifications of RNP structure. Since RNPs differ from naked RNAs in net charge and molecular weight, we investigated the effect of tubulin on YB-1:RNA complexes by electrophoretic mobility gel-shift assay. It is known that in saturated mRNPs one molecule of YB-1 is bound to 25 RNA bases (Skabkin *et al.*, 2004;Chernov et al., 2008a). Compared to RNA alone, these saturated YB-1:RNA complexes demonstrate a significant reduction of mobility in native agarose gel due to either partial RNA discharging or the increased mass of the formed YB-1:RNA complex, or both (Figure 21, compare lane 1 and 3). The presence of tubulin did not change the mobility of RNA that excluded the possibility of the direct interaction between these two molecules (compare lanes 1 and 2). The addition of tubulin to YB-1:RNA complexes clearly increased the mobility of RNP (compare lane 3 with 4 and 5). The presence of tubulin may thus induce structural changes of RNP complexes and/or a change in the RNP charge via YB-1 withdrawal mediated by tubulin.

In conclusion, we demonstrated that:

- YB-1 interacts directly with tubulin and microtubules.
- The interaction between YB-1 and microtubules is partly electrostatic and in part the result of molecular recognition.
- YB-1 promotes the assembly of normal microtubules *in vitro.*
- Tubulin interferes with formation of mRNPs.

D.2. STRUCTURE OF mRNPs AND mRNPs: MICROTUBULES INTERACTION (PUBLICATIONS II AND III)

As mentioned above, polysomal mRNPs contain twice less YB-1, than silenced mRNPs. The aim of this study was to define a link between translational status of RNPs and their structure. To form mRNP complexes, purified YB-1 and mRNA were incubated at different molar ratios (paper II). Compared to α-globin mRNA alone which sediments as 9S particle, YB-1:RNA complexes formed at base ratio of 1/55 possess sedimentation coefficient of 18S. Further addition of YB-1 to R=1/18 increases sedimentation coefficient of complexes up to 28S (Figure 22). Higher amounts of YB-1 did not increase the sedimentation coefficient of RNP that indicated saturation of complexes at R=1/18. The appearance of some unbound YB-1 before RNA saturation could be explained by partial dissociation of protein from RNP during centrifugation. The sedimentation coefficient and buoyant density of saturated mRNP were 28S and 1.39 g/cm^3 respectively, which are typical for natural untranslated α-globin mRNP (Preobrazhensky and Spirin, 1978). These results suggest that properties of reconstructed and natural mRNPs depend on YB-1:RNA base ratio and that addition of YB-1 to mRNA caused gradual increase in the level of RNP compaction.

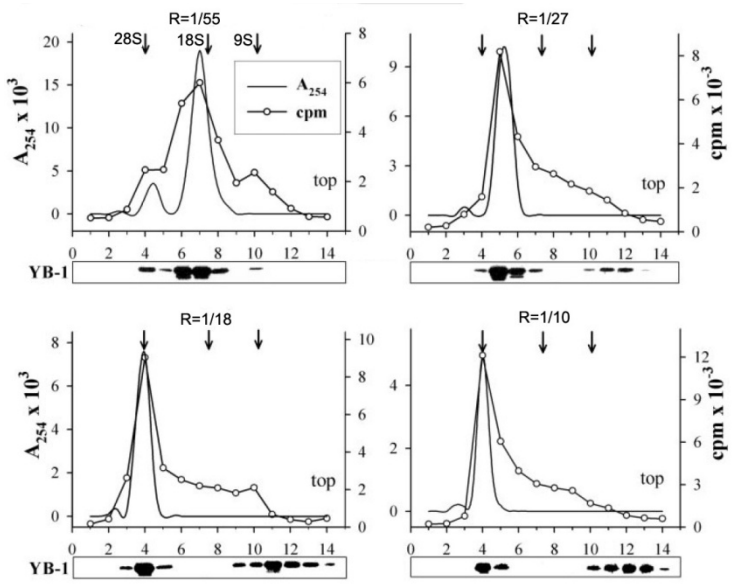


Figure 23: Sedimentation analysis of YB-1: α-globin mRNA complexes. From Skabkin et al., 2004.

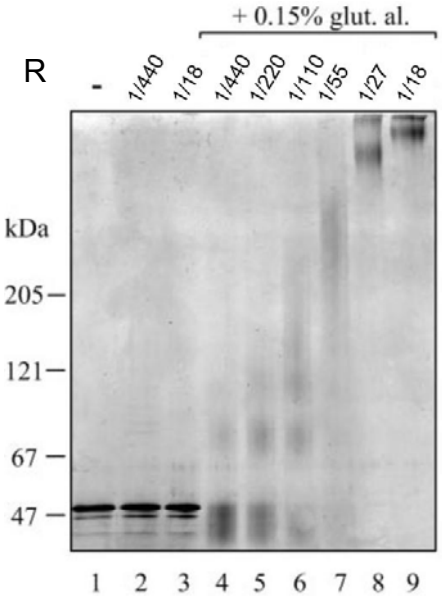


Figure 22: Dependence of YB-1 oligomeric state on R ratio. From Skabkin et al., 2004.

Several independent groups reported that free YB-1 exists in homo-oligomeric form with molecular mass of 800 kDa and sedimentation coefficient of 18S (Evdokimova *et al.*, 1995;Gaudreault et al., 2004). However question arises whether YB-1 is associated with mRNA in multimeric or monomeric form. To explore this issue we performed biochemical analysis of unsaturated and saturated RNP complexes. RNP complexes were formed at different R ratios and cross-linked with 0.15% glutaraldehyde. To investigate sizes of protein complexes, we digested RNA with RNAses and analyzed cross-linking products by 4-15% SDS-PAGE. As seen on Figure 23, at low R ratios protein appears as monomer (lanes 4 and 5), whereas an increase of the R ratio caused the appearance of oligomeric protein forms of gradually increasing size (Figure 23, lanes 6, 7, 8 and 9). Within saturated RNP complexes (R=1/18) all YB-1 was found in huge oligomeric globules with molecular mass exceeding the highest marker (205 kDa). This result supported the proposal that structure of RNP changes upon increasing the YB-1:RNA ratio.

Complementary results were obtained from AFM imaging of YB-1:2Luc mRNA complexes, formed at different R ratios. This synthetic mRNA was unusually long (3000 bases) that allowed investigation of RNP ultrastructure via high resolution imaging. First, we formed unsaturated RNP complexes at R ratio of 1/200 and fixed them with 0.15% glutaraldehyde before adsorption on APS-mica surface (Figure 24). Unsaturated complexes consisted of several protein globules with heights below 2 nm and appeared quite elongated and unfolded. In contrast, saturated RNP (R=1/12) appeared as compacted beads-on-string structures contained globular particles with mean height of 7 nm. This observation again supports the conclusion that YB-1 undergoes multimerization upon mRNA saturation (Figure 24).

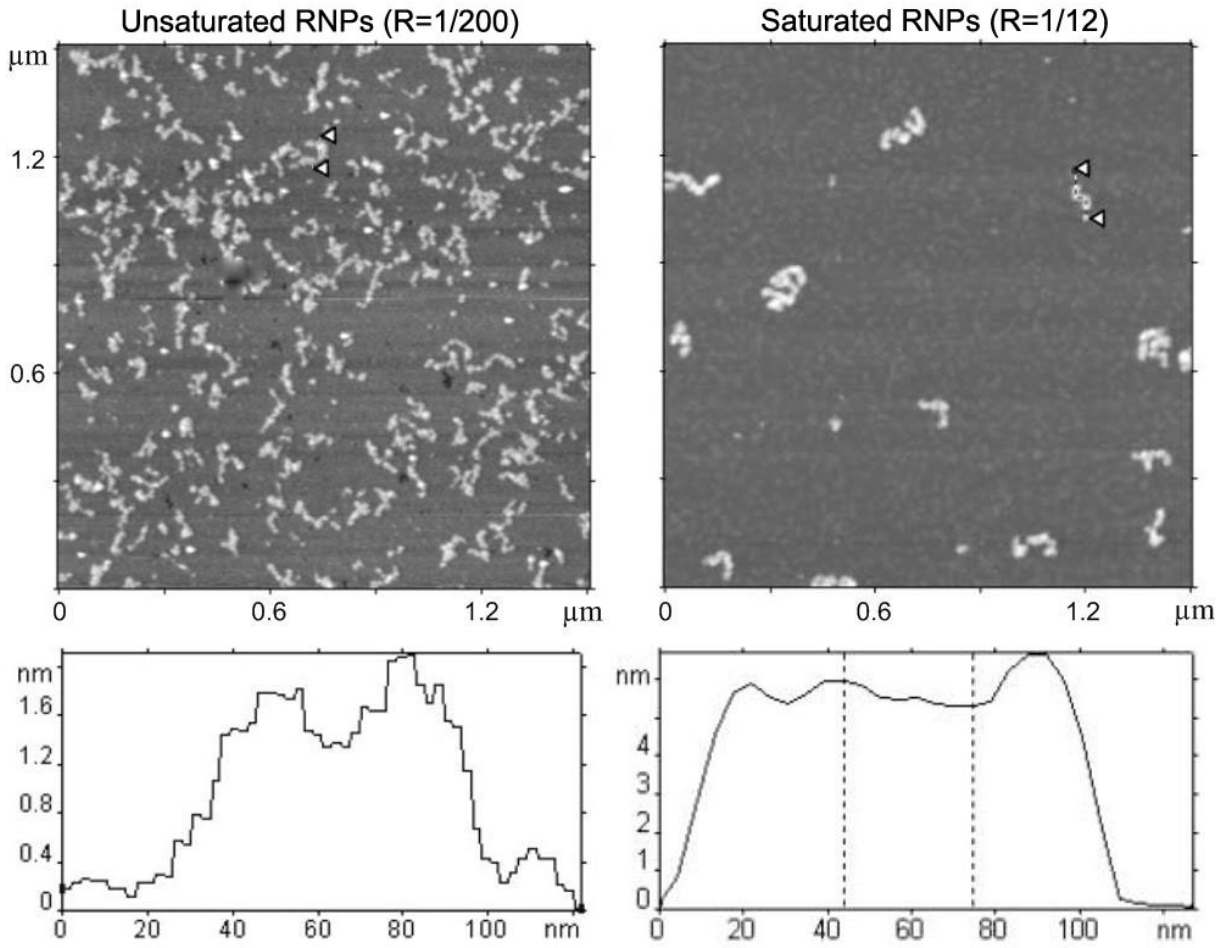


Figure 24: AFM images and cross-sections of unsaturated and saturated 2Luc RNPs. From Skabkin et al., 2004.

As reported above, we characterized tubulin as a novel YB-1-interacting protein and analyzed in details YB-1:tubulin interaction (Chernov et al., 2008b). Recently, large scale identification of tubulin and microtubule binding proteins showed that 21% of them were known mRNA binding proteins like PABP (Chuong et al., 2004). It was also shown that, in the cytoplasm, mRNP may associate with and be transported along microtubules by molecular motors (Palacios and St Johnston, 2001). Several cellular and viral proteins play dual roles joining translational regulation with cytoskeleton (see A.4. for details). As Y-box proteins are major mRNP component in many types of cells (Deschamps et al., 1992;Davydova et al., 1997;Yu et al., 2002), they are good candidates to participate in the processes of mRNA trafficking, protection and anchoring of mRNA to cytoskeleton. Thus, we asked whether YB-1 could mediate the attraction of mRNA to the cytoskeleton, particularly to

microtubules. In agreement with previous results, we found that saturated mRNP particles (R=1/10) possess a beads-on-string structure, whereas mRNA alone appears flat and more spread on the mica surface (Figure 25, panels A and B). After addition of pre-formed taxol-stabilized microtubules almost all mRNPs were found in close proximity to microtubules, whereas mRNA alone didn't interact with them (Figure 25, panels C and D). These observations allowed us to conclude that YB-1 promotes the binding of mRNPs to microtubules.

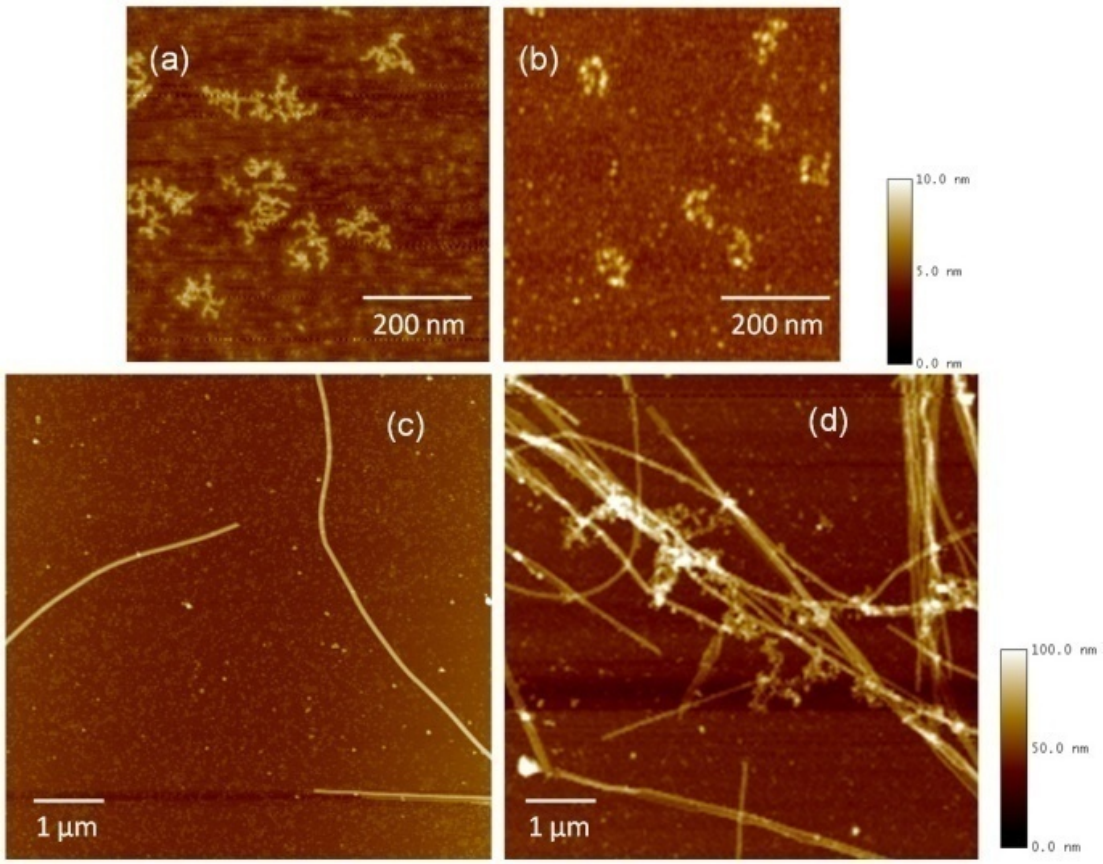


Figure 25: High resolution AFM imaging of RNPs: microtubule association: 2 Luc mRNA appeared spread and less compacted (a), than saturated RNP complexes (b). Microtubules and mRNA did not significantly interact with each other (c), whereas RNPs clearly coat microtubules (d).

We also found that all RNPs co-sedimented with microtubules at low ionic strength (I=50 mM), whereas increase of the ionic strength provoked gradual release of RNPs from microtubules. Notably, RNPs remained bound to microtubules at concentrations of KCl slightly higher than physiological. This result suggests that regardless its electrostatic origin RNP:microtubule interaction persists at physiological concentration of salt and thus could be significant *in vivo*. To explore the mechanism of RNP:microtubule attraction, we performed non-denaturing gel electrophoresis of complexes formed at different R ratios, varying from 1/30 (non-saturated mRNP) to 1/7.5 (saturated mRNP). The interaction between RNPs and microtubules was then analyzed by 0.6 % agarose non-denaturing gel electrophoresis (Figure 26). We found that addition of tubulin or microtubules didn't change the electrophoretic mobility of mRNA alone, indicating that it interacts neither with tubulin nor with microtubules. Addition of YB-1 resulted in a gradual decrease of RNA mobility due to either neutralization of its negative charge or to an increase in the size of the complex. In contrast, the presence of tubulin increased the mobility of the complex reflecting a competition between tubulin and mRNA for YB-1 binding.

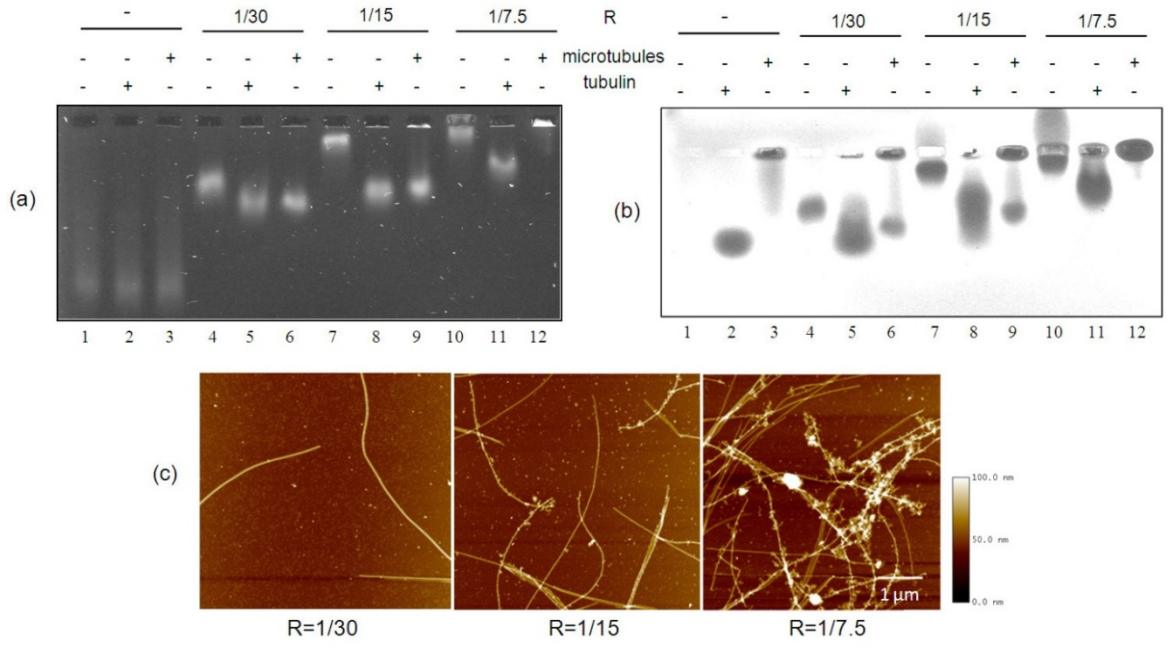


Figure 26: YB-1 promotes mRNA binding to microtubules at high R values (R≥1/15).

Upper panel: mRNA was incubated alone (lanes 1–3) or with varying concentrations of YB-1 (lanes 4–12). After incubation with YB-1, tubulin or microtubules were added and samples were analyzed by native agarose gel electrophoresis. (a) RNA detection with Sybr Green II. (b) Proteins detection with Coomassie blue staining.
Lower panel: (c) AFM imaging of RNPs:microtubules association at various R values.

Similar effects were produced by the addition of taxol-stabilized microtubules at R=1/30 and R=1/15. However, at R=1/15 a small fraction of RNPs was associated with microtubules and remained in the well, whereas at R=1/7.5 all RNP were bound to microtubules (Figure 26).

AFM imaging provided information in agreement with these results. We did not observed any RNP at the microtubule surface at R=1/30, whereas at R=1/15 a fraction of RNPs was found to be associated with microtubules. At high R value of 1/7.5 almost all RNPs were found in close association with microtubules. Above saturation we also observed the presence of free YB-1 (migration toward cathode) (Figure 26 B, lanes 7 and 10). Interestingly, in these conditions, RNPs were associated in large aggregates. From these observations and our knowledge of YB-1 we hypothesize that extra YB-1 molecules are bound to microtubules and RNPs and thus available for protein-protein interaction via their C-terminal multimerization domains. This prediction in indeed in accordance with previous results, stating that the C-terminal domain of YB-1 can be displaced from RNA by “cold-shock” domain at high R values (Skabkin *et al.*, 2004). Another possibility is the formation of salt bridges between oppositely charged C-termini of YB-1 and tubulin (Figure 27).

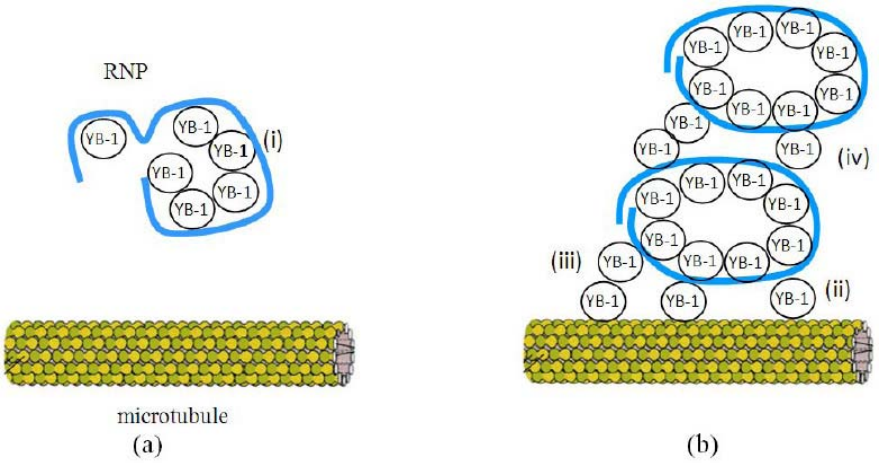


Figure 27: Model of RNA:microtubule attraction mediated by cationic mRNA binding proteins. (a) There is no attraction between non-saturated RNP and microtubules. (b) Extra proteins bound to microtubules and RNAs mediate RNP:microtubule attraction via formation of salt bridges (ii) or protein:protein interaction (iii). From Chernov et al., 2008a.

Finally, to extend our finding, we evaluated the possibility that PABP, another major core RNP protein can also mediate RNA:microtubule binding. We observed by AFM that PABP also provoke such a binding and triggered the formation of huge aggregates on microtubule surface. This microtubule-dependent aggregation could

occur during the stress response when cells protect themselves against toxic environmental conditions (Ivanov et al., 2003b). Thus, other mRNA binding proteins containing oligomerization domains demonstrate similar properties to YB-1. The possible significance of our observations is highlighted by the fact that Y-box proteins are very abundant cellular component. One of the most striking examples is MSY2 that was found in cytoskeleton-associated granular structures close to oocyte cortex (Yu *et al.*, 2001). MSY2 is the major oocyte RNA-binding protein that packs mRNA into inactive mRNP. It has been estimated that each oocyte contains about 80 pg of total mRNA and 0.49 ng of MSY2 protein that gives R=1/15. Thus, all RNPs contain saturated amounts of MSY2 which potentially can bridge mRNA with microtubules. Altogether our data open new perspectives in studying of mRNA association with cytoskeleton via cationic proteins that can take place both *in vitro* and in living cells.

In conclusion, we demonstrated that:

- Repression of mRNA translation is probably caused by the change of mRNP structure.
- At high YB-1:mRNA base ratios huge protein oligomeric globules increase level of mRNP compaction.
- Cationic proteins can make a bridge between mRNP and microtubules *in vitro.*
- Microtubules provide a surface that stimulates the process of mRNP aggregation.

D.3. MECHANISM OF YB-1-INDUCED TRANSLATION INHIBITION (PUBLICATION IV)

It has been shown that the translational status of YB-1 containing mRNPs depends on the YB-1:mRNA base ratio. However, the precise stage of translation affected by YB-1 remained to be elucidated. To address this issue we investigated the effect of YB-1 in cell-free translational system more deeply. As previously, we found that the addition of YB-1 caused a dose-dependent inhibition of endogenous and exogenous globin mRNA translation. This inhibition was accompanied by a rapid polysomal decay and an accumulation of mRNA in the form of free mRNP. Extraction of mRNA from the translation mixture revealed that YB-1 didn't provoke mRNA degradation or its functional inactivation. Similarly, YB-1 didn't interfere with formation of 43S preinitiation complexes. A measure of time required for the release of nascent polypeptide from the ribosomes indicated also shown an insensitivity of elongation and termination steps to the presence of YB-1. These results indicate that YB-1 interferes rather with earlier steps of translation. Initiation of translation in eukaryotes is a complex multistep process, beginning with joining of 40S subunit to mRNA mediated by eIF4E, eIF4G and eIF3. A central role in this process plays eIF4G that act as a scaffold protein which makes a bridge between cap structure of mRNA and small ribosomal subunit. We found that YB-1-induced translation inhibition correlated with displacement of eIF4G from globin mRNA in rabbit reticulocyte extract. The ability of YB-1 to displace eIF4F was also confirmed by experiments with isolated components. The study of truncated proteins revealed that the C-terminal domain and the entire YB-1 disrupted interaction of eIF4G with mRNA and thus suppressed cap-dependent translation initiation, whereas the AP-CSD domains were unable to displace this factor and didn't affect translation. Therefore, the binding of YB-1 to mRNA via its C-terminal domain provokes removal of eIF4G, disruption of mRNA:40S subunit interaction and inhibition of translation at the initiation stage. On the other hand, the increase of eIF4G concentration leads to a displacement of YB-1 from 5' UTR and translational activation. Together our results show that competition between eIF4G and YB-1 for mRNA binding determines its translational status and stability both *in vitro* and within the cell.

D4. FACILITATED DIFFUSION AS A SOLUTION TO THE PROBLEM OF DIFFUSION-LIMITED TUBULIN ASSEMBLY

(SUBMITTED PAPER)

Microtubule dynamics is a highly complex and regulated process which depends on multiple factors. While many different models were successfully proposed to explain experimental data, the mechanism of tubulin polymerization remains under discussion (Voter and Erickson, 1984b;Flyvbjerg et al., 1996b;Odde, 1997b;Caudron et al., 2000;Antal et al., 2007;Zhao and Sokhansanj, 2007;Rezania et al., 2008). One interesting question concerning tubulin polymerization remains unanswered: is the diffusion of tubulin subunits to microtubule ends a potential rate-limiting step? If we only consider the calculated rate of encounter of tubulin with microtubule ends, it appears at first glance that the response is no. Indeed, Smoluchowski equation used to calculate Js, the rate of encounters between tubulin heterodimers and a body of radius b_0 (*nucleus or MT ends*), gives a value of about 1500-3800 collisions per second[1] which seems to be largely enough for typical elongation rates (about *14 µm/min, with ~1640 tubulin subunits per µm (Odde, 1997c)*). Indeed, if we neglect treadmilling, the association rate of tubulin to one end needs to be only larger than 190 s^{-1} to reach 14 µm/min. This conclusion is however in apparent contradiction with at least three experimental evidences: i) microtubule collapse upon dilution (Walker et al., 1991), ii) near critical concentration, the tubulin supply is so critical that elongation only compensate shortening (Walker et al., 1988b), iii) elongation rate depends on tubulin concentration, the larger is the free tubulin concentration, the faster it is (Walker et al., 1988a), iv) the rate of tubulin nucleation also decreases sharply with free tubulin concentration (Voter and Erickson, 1984a;Leguy et al., 2000), v) finally, experimental measures of assembly rates at given tubulin concentration both *in vivo* and *in vitro* are ~ 0.03-0.1 µm/s (Mitchison *et al.*, 1984;Cassimeris et al., 1988). Thus, only one collision on 100-300 seems effective for the incorporation of a new tubulin subunit into microtubule. At least two physical

[1] $J_s=4\pi D_3 C_0 b_0$, where D_3 is the 3D diffusion coefficient of tubulin, b_0 is the size of the nucleus of the MT ends and C_0 is the concentration of free tubulin. The value $b_0 \approx 4$-10nm (4 nm is the value previously used to describe the tip size at the MT ends (Odde, 1997a)), $D_3 \approx 5.10^{-12}$ m²/s and $C_0=10$ µM (measured in sea urchin egg extracts (Salmon et al., 1984)).

parameters counteract the incorporation of tubulin in microtubule: i) electrostatic repulsion between the negatively charged microtubule surface and tubulin, ii) geometrical factor that requires a proper orientation of tubulin for association with growing nucleus or tips of microtubules. The highly negatively charged C-termini of tubulin subunits are exposed on the microtubule surface in proximity to each other and this causes subunits repelling. At the beginning of its journey to the microtubule wall, an incoming tubulin molecule first interacts with the nucleus/microtubule extremity in a chaotic orientation. The probability that its orientation is different from that observed in the microtubule is however very high and thus a proper orientation of the tubulin molecule is required by rotational diffusion following the initial interaction. This concept is supported by the fact that tubulin polymerization is enhanced in buffers containing glycerol and chaotropic agents like guanidine hydrochloride, where hydrophobic aggregation is favored (Wolff et al., 1996). Together these facts could indicate that diffusion is limiting step in the process of tubulin assembly. Furthermore it is also well known that different positively charged molecules favor tubulin polymerization, and it was advanced that they act by reducing electrostatic repulsion between like-charged tubulin molecules (Erickson and Voter, 1976c;Mithieux et al., 1984b;Wolff, 1998b). It appears thus that both geometrical factor and electrostatic repulsion may be interconnected and that the requirements for a proper tubulin orientation for molecular recognition may be very strict in the process of tubulin assembly. We propose that small positively charged molecules may facilitate the re-orientation process, by avoiding close contact between like charged C-termini and also participate to an attraction force between tubulin molecules (either free or in the microtubules) via charge sharing.

Natural polyamines, like spermidine and spermine, are present at micromolar concentrations within cells where they mainly interact with nucleic acids. It is however considered that more than 100 μM are free of nucleic acids (Igarashi and Kashiwagi, 2000). Although the enhancement of tubulin polymerization by polyamines has been described, the detailed mechanism of their action still remains elusive. We hypothesized that these molecules could increase tubulin polymerization not only due to the decreasing of electrostatic repulsion between tubulin molecules but also thanks to an enhanced “facilitated diffusion” along microtubules due to polyamines sharing by tubulin.

Facilitated diffusion was proposed many years ago for transcription factors. Indeed, transcription factors are able to find their binding sites 100 times faster than expected using a 3D diffusion model. This obvious discrepancy between theoretical and experimental data was resolved by introducing a facilitated diffusion mechanism in which transcription factors first bind to DNA and then slide along DNA by 1D diffusion. Thus, the time to find a specific DNA sequence by 1D sliding is less, than by 3D diffusion. Facilitated diffusion of transcription factors is possible thanks to their positive charge and non-specific affinity for DNA. How such an attraction can occur between two negatively charged compounds, like the tubulin molecule and the microtubule surface? The sharing of multivalent cations may provide such a link and allow the sliding of tubulin on microtubule surface.

In our laboratory, Dr. David Pastré successfully modelled the consequences of polyamine sharing on microtubule growth rate taking into the account possibility of facilitated tubulin diffusion on microtubule surface. This mechanism helps to understand apparent contradictions related to persistent microtubules assembly under diffusion limited conditions.

The model can predict the impact of facilitated diffusion for microtubule nucleation and elongation processes. Results of modeling for different lengths of microtubules and attraction energies are presented on Figure 28. The rate of tubulin reaching MT ends via facilitated diffusion versus microtubule length was determined for four attraction energies, (*U_c=-0.1 K_BT, very low; U_c=-1 K_BT, low; U_c=-2.5 K_BT, moderate; U_c=-6 K_BT, very strong*). As seen on Figure 28 A, facilitated diffusion increases significantly the flux of protein arriving to the MT ends for low and moderate attractions (*for L~1µM, $J_{facilitated}$ increase about 4 times in the cases of Uc=-0.1 K_BT and -2.5 K_BT)* but is not beneficial for high attraction (*Uc=-6 K_BT)*. A strong attraction should induce shorter microtubules since the flux of tubulin is significantly smaller for longer microtubules. Regarding microtubule assembly curve versus time we observe that facilitated diffusion both increases the plateau value and the steepness of the assembly curve (Figure 28 B).

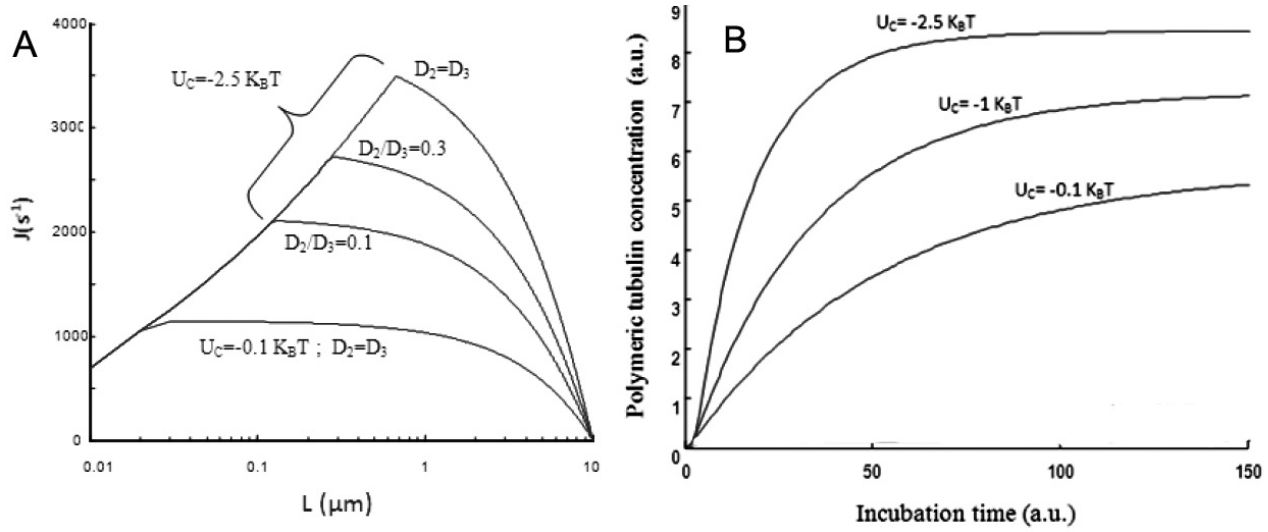


Figure 28: A. Facilitated diffusion of free tubulin to microtubule ends versus mean microtubule length for different absorption energies (U_c). B. Model of microtubule assembly versus time for different attraction energies, which highlights the influence of facilitated diffusion on the microtubule growth.

Regarding nucleation, the model predicts that if the time lapse between two consecutive encounters is shorter than the lifetime of the tubulin:nucleus interaction per encounter, the association constant should weakly depend on the initial tubulin concentration. This occurs above a concentration designated C_L (Figure 29, *left panel*) below which the association rate decreases more abruptly. This latter feature is typical for facilitated diffusion.

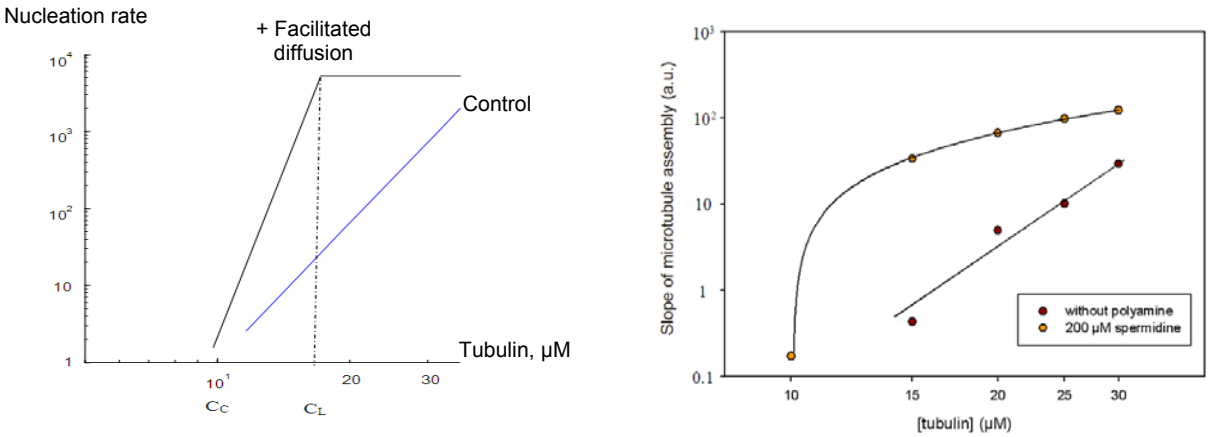


Figure 29: In the presence of facilitated diffusion, the rate of nucleation is weakly dependent on the tubulin concentration over concentration C_L. However, at concentrations lower than C_L, the lifetime of tubulin adsorption on the nucleus is lower than the time between two consecutive collisions, the nucleation rate then depends on tubulin concentration. *Left panel*: theoretical prediction. *Right panel*: experimentally obtained result.

To confirm our theoretical findings, we measured the influence of poylamines on the maximum slope of the microtubule assembly curve (Figure 29, *right panel*). We found that at high concentrations of spermidine, the rate of microtubule assembly depends weakly on tubulin concentration whereas it increases linearly with tubulin concentration in the absence of polyamine where the slope is about 6, in agreement with previous estimation of the nucleus size (~ *12 tubulin heterodimers*). In the presence of spermidine, the nucleation rate exhibits two regions with a weak dependence on tubulin concentration over the C_L concentration (*10-15 µM*) typical of a facilitated nucleation process (Figure 29, *left panel*).

In a second part of the work, we further tested experimentally the validity of this model *in vitro* using purified tubulin or tubulin-MAPs and different kind of polyamines. The results show that the promotion of microtubule assembly by polyamines is typical of facilitated diffusion both on the nucleation and elongation steps in agreement with the model. Figure 30 shows the effect of different type of polyamines on the rate of microtubule assembly. At tubulin concentration near the critical concentration, we observed that spermidine (3+) and spermine (4+) promote polymerization at submilimolar concentrations whereas putrescine (2+) had no effect. At high concentrations of spermidine and spermine (>400 µM), the assembly rate was lowered leading to a bell-shape profile as predicted by the model. The inset shows that digestion of tubulin with subtilisin relieves the effect of polyamines.

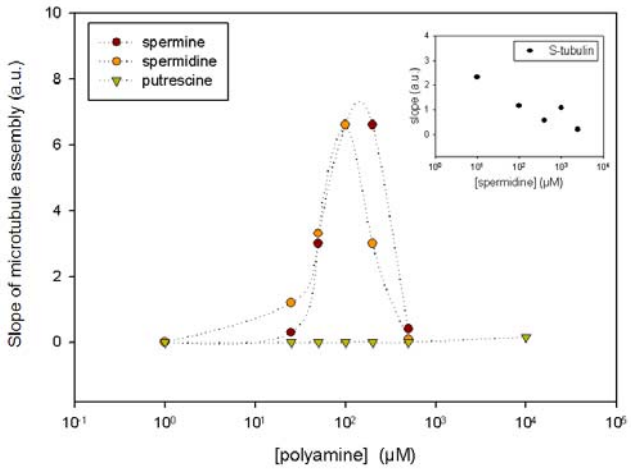
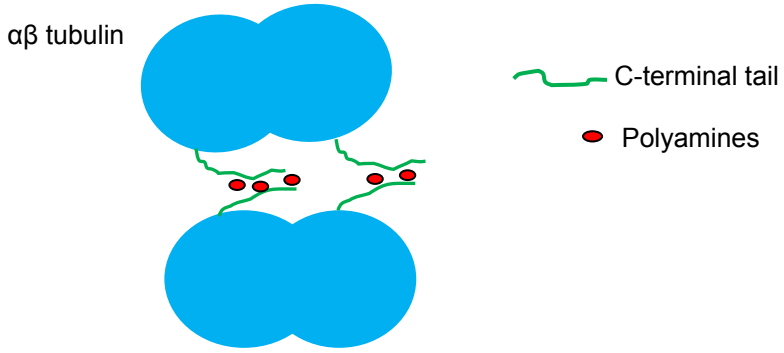


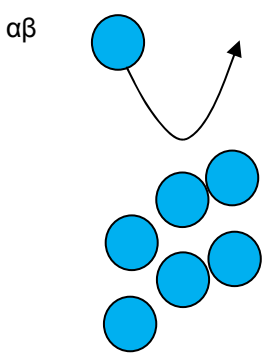
Figure 30: The effect of different natural polyamines on microtubule assembly.

The following figure summarizes the findings (Mechulam et al., 2009):

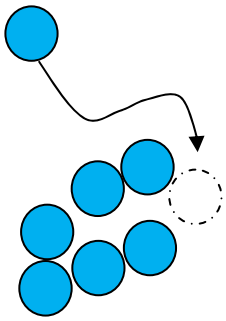
A - Polyamine sharing creates an attraction force between tubulin molecules.



B - Attraction force facilitates nucleation.

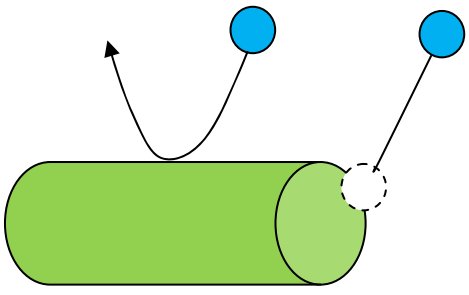


Collision and dissociation in the absence of an attraction force.

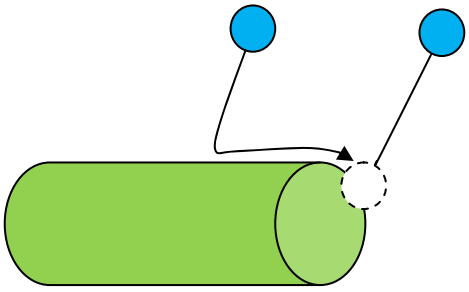


Collision, surface diffusion and association to the nucleus in the presence of an attraction force.

C - Facilitated diffusion of tubulin favors microtubule assembly.



Without facilitated diffusion, only tubulin which encounters directly the ends of the MT in a correct orientation could be incorporated.



Facilitated diffusion allows the sliding of tubulin along the MT wall and favours tubulin association to the MT ends.

In conclusion, our analytical model and experimental data demonstrated that:

- Facilitated diffusion can actually influence microtubule assembly.
- Facilitated diffusion increases nucleation rate for low tubulin concentrations, speed of microtubule growth and steady-state mass of the polymer for intermediate attraction energies and microtubule lengths.
- Polyamine sharing by tubulin is one mechanism by which facilitated diffusion may influence microtubule dynamics.

E. CONCLUSION AND PERSPECTIVES

YB-1 is a multifunctional protein that was well known as a key regulator of transcription and translation due to its interaction with nucleic acids. Interestingly, this protein was also found to interplay with other cellular components, including numerous protein partners, such as actin, splicing factors, p53, DEAD helicases and RNA polymerase II. In the present work we extend the spectrum of YB-1 protein partners and show that YB-1 may interact with tubulin and microtubules acting as a regulator of microtubule dynamics. Moreover, our findings provide multiple putative links between cytoskeleton dynamics and mRNA metabolism. Obtained results open interesting perspectives in different fields :

INTERPLAY OF YB-1 BETWEEN TUBULIN/MICROTUBULES AND mRNA

Our work led to the description of YB-1 as a novel tubulin and microtubule partner *in vitro* and to the understanding of its impact on microtubule dynamics. We therefore ask the question of its involvement in the regulation of microtubule dynamics in living cells. Up to now, we didn't get any proof supporting the interaction between YB-1 and tubulin/microtubules *in vivo*. Intracellular co-localization and co-sedimentation of proteins from cellular extracts could provide evidences supporting the idea of interaction between these proteins. We performed immunostaining of different cell types with anti-YB-1 and anti-tubulin antibodies but didn't obtain co-localization between YB-1 and microtubules. Similarly, YB-1 did not co-sediment with taxol-stabilized microtubules from HeLa cell extracts that also argues against the possibility of YB-1/microtubule interaction *in vivo*. However, an independent group documented the localization of YB-1 within the centrosome in mitotic cells and proposed YB-1 as a new microtubule nucleating factor (Janz et al., 2000). These data are still waiting to be confirmed by other laboratories. Growth stimuli promotes release of YB-1 from mRNA due to phosphorylation by Akt kinase (Bader and Vogt, 2008b). Partial proteasomal degradation of YB-1 could also modify its RNA-binding properties and provoke protein translocation into nucleus (Sorokin et al., 2005). Altogether, post-translational modification of YB-1 could release protein from mRNPs and make it available for protein:protein interactions. These facts indicate that YB-1 could sequester tubulin in a non-polymerized form under certain physiological conditions.

To shed light on this issue we could perform co-immunoprecipitation of YB-1 and tubulin from different sources.

In the pathology of Alzheimer's desease, the microtubule associated Tau protein becomes detached from microtubules and forms huge fibrillar structures by still discussed mechanism (Delacourte and Defossez, 1986). Probably, this detachment makes microtubule surface available for the interaction with other cellular components and interferes with axonal transport of different cargoes, including mRNP particles. Further investigations of Alzheimer's desease requires analysis of intracellular localization of mRNP proteins under normal and pathological conditions. In this perspective, YB-1 and other mRNP components could be possible targets for the treatment of neurodegenerative deseases.

Recently, the interaction of mRNPs with microtubules has been demonstrated in Xenopus laevis and mitotic HeLa cell extracts (Blower et al., 2007). Only a sub-fraction of mRNAs, which encodes proteins involved in the regulation of cell cycle, has been co-purified with mitotic microtubules. This pool of microtubule-bound mRNAs was locally translated onto microtubules during mitosis, suggesting that localized translation might be a key regulator of cell cycle. The modulation of cell-cycle dependent mRNP binding to microtubules and inhibition of their translation may be used as a future strategy for the treatment of cancer.

Several stresses, such as heat-shock, oxidative stress and treatments with drugs provoke translational arrest, rapid polysome disassembly and compartmentalization of cellular translational apparatus into huge aggregates (Anderson and Kedersha, 2008b). This protective mechanism of protein biosynthesis repression helps cells to keep energy for survival, because only few specific proteins (such as heat-shock proteins) are expressed during stress. Moreover, stress granules could serve as sites for cellular mRNPs protection against damaging environment. It has been shown that several proteins involved in global mRNA metabolism, may play auxiliary «emergency» functions during stress. For example, the mRNP proteins TIA and TIAR, normally localized into nucleus, migrate into the cytoplasm in response to stress where they nucleate granule formation (Taupin et al., 1995;Gilks et al., 2004). Recent cellular observations identified YB-1 and PABP as ubiquitous components of stress granules (Kedersha *et al.*, 2007). It has been shown also that disruption of

microtubule array with nocodazole or vinblastine abolished arsenate-induced formation of stress granules (Ivanov et al., 2003a). These interesting findings, taken together, indicate an involvement of microtubule dynamics in cell survival under translational arrest. The possibility of a synergism between microtubule-targeting drugs and translation inhibitors open possibilities for development of new anti-cancer strategies.

FACILITATED DIFFUSION AS A MECHANISM OF MICROTUBULE DYNAMICS

It has been known for more than 20 years that natural polyamines play important roles in cellular division (Pohjanpelto et al., 1981b;Anehus et al., 1984). Polyamine concentration changes during the cell cycle, with two significantly pronounced peaks at the G1/S and S/G2 transitions that were related to their involvement in processes of DNA synthesis (Oredsson, 2003). In addition, polyamine starvation of cells drastically altered microtubule and actin networks, whereas it had no effect on intermediate filaments. These facts suggest a selective interaction between polyamines and negatively charged cellular cytoskeletal elements (Pohjanpelto et al., 1981a). Multiple cellular effects of polyamines indicate also an exceptional role of polyamines for cellular metabolism and open perspectives for the treatment of cancer via modulation of intracellular polyamine levels. Selective inhibition of ornithine decarboxylase which is a central enzyme of polyamine biogenesis together with blockage of polyamine transporters can potentially be used for anti-cancer therapy (Pegg, 2006). In addition, we proposed a novel mechanism explaining the positive effect of these molecules on the microtubule network. This opens the possibility to directly target the interaction of polyamines with tubulin and microtubules by small pharmacological compounds.

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Evry, December 2008

Konstantin Chernov

G. REFERENCES

Amos,L.A. and Schlieper,D. (2005). Microtubules and maps. *Adv. Protein Chem.,* **71,** 257-298.

Anderson,P. and Kedersha,N. (2006). RNA granules. *J. Cell Biol.,* **172,** 803-808.

Anderson,P. and Kedersha,N. (2008b). Stress granules: the Tao of RNA triage. *Trends Biochem. Sci.,* **33,** 141-150.

Anderson,P. and Kedersha,N. (2008a). Stress granules: the Tao of RNA triage. *Trends Biochem. Sci.,* **33,** 141-150.

Anehus,S., Pohjanpelto,P., Baldetorp,B., Langstrom,E., and Heby,O. (1984). Polyamine starvation prolongs the S and G2 phases of polyamine-dependent (arginase-deficient) CHO cells. *Mol. Cell Biol.,* **4,** 915-922.

Ansari,S.A., Safak,M., Gallia,G.L., Sawaya,B.E., Amini,S., and Khalili,K. (1999). Interaction of YB-1 with human immunodeficiency virus type 1 Tat and TAR RNA modulates viral promoter activity. *J. Gen. Virol.,* **80 (Pt 10),** 2629-2638.

Antal,T., Krapivsky,P.L., Redner,S., Mailman,M., and Chakraborty,B. (2007). Dynamics of an idealized model of microtubule growth and catastrophe. *Phys. Rev. E. Stat. Nonlin. Soft. Matter Phys.,* **76,** 041907.

Ashizuka,M., Fukuda,T., Nakamura,T., Shirasuna,K., Iwai,K., Izumi,H., Kohno,K., Kuwano,M., and Uchiumi,T. (2002). Novel translational control through an iron-responsive element by interaction of multifunctional protein YB-1 and IRP2. *Mol. Cell Biol.,* **22,** 6375-6383.

Bader,A.G. and Vogt,P.K. (2008b). Phosphorylation by Akt disables the anti-oncogenic activity of YB-1. *Oncogene,* **27,** 1179-1182.

Bader,A.G. and Vogt,P.K. (2008a). Phosphorylation by Akt disables the anti-oncogenic activity of YB-1. *Oncogene,* **27,** 1179-1182.

Belmont,L.D. and Mitchison,T.J. (1996). Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. *Cell,* **84,** 623-631.

Bhattacharyya,B., Sackett,D.L., and Wolff,J. (1985). Tubulin, hybrid dimers, and tubulin S. Stepwise charge reduction and polymerization. *J. Biol. Chem.,* **260,** 10208-10216.

Blobel,G. (1972). Protein tightly bound to globin mRNA. *Biochem. Biophys. Res. Commun.,* **47,** 88-95.

Blobel,G. (1973). A protein of molecular weight 78,000 bound to the polyadenylate region of eukaryotic messenger RNAs. *Proc. Natl. Acad. Sci. U. S. A,* **70,** 924-928.

Blower,M.D., Feric,E., Weis,K., and Heald,R. (2007). Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules. *J. Cell Biol.,* **179,** 1365-1373.

Bokros,C.L., Hugdahl,J.D., Kim,H.H., Hanesworth,V.R., van Heerden,A., Browning,K.S., and Morejohn,L.C. (1995). Function of the p86 subunit of eukaryotic initiation factor (iso)4F as a microtubule-associated protein in plant cells. *Proc. Natl. Acad. Sci. U. S. A,* **92,** 7120-7124.

Boyko,V., Ferralli,J., Ashby,J., Schellenbaum,P., and Heinlein,M. (2000). Function of microtubules in intercellular transport of plant virus RNA. *Nat. Cell Biol.,* **2,** 826-832.

Braddock,M., Muckenthaler,M., White,M.R., Thorburn,A.M., Sommerville,J., Kingsman,A.J., and Kingsman,S.M. (1994). Intron-less RNA injected into the nucleus of Xenopus oocytes accesses a regulated translation control pathway. *Nucleic Acids Res.,* **22,** 5255-5264.

Bulinski,J.C. and Borisy,G.G. (1980). Microtubule-associated proteins from cultured HeLa cells. Analysis of molecular properties and effects on microtubule polymerization. *J. Biol. Chem.,* **255,** 11570-11576.

Caplow,M. and Shanks,J. (1996). Evidence that a single monolayer tubulin-GTP cap is both necessary and sufficient to stabilize microtubules. *Mol Biol Cell,* **7,** 663-675.

Cassimeris,L., Pryer,N.K., and Salmon,E.D. (1988). Real-time observations of microtubule dynamic instability in living cells. *J. Cell Biol.,* **107,** 2223-2231.

Castoldi,M. and Popov,A.V. (2003). Purification of brain tubulin through two cycles of polymerization-depolymerization in a high-molarity buffer. *Protein Expr. Purif.,* **32,** 83-88.

Caudron,N., Valiron,O., Usson,Y., Valiron,P., and Job,D. (2000). A reassessment of the factors affecting microtubule assembly and disassembly in vitro. *J. Mol. Biol.,* **297,** 211-220.

Cheng,S. and Gallie,D.R. (2007). eIF4G, eIFiso4G, and eIF4B bind the poly(A)-binding protein through overlapping sites within the RNA recognition motif domains. *J. Biol. Chem.,* **282,** 25247-25258.

Chernov,K.G., Curmi,P.A., Hamon,L., Mechulam,A., Ovchinnikov,L.P., and Pastre,D. (2008a). Atomic force microscopy reveals binding of mRNA to microtubules mediated by two major mRNP proteins YB-1 and PABP. *FEBS Lett.,* **582,** 2875-2881.

Chernov,K.G., Mechulam,A., Popova,N.V., Pastre,D., Nadezhdina,E.S., Skabkina,O.V., Shanina,N.A., Vasiliev,V.D., Tarrade,A., Melki,J., Joshi,V., Baconnais,S., Toma,F., Ovchinnikov,L.P., and Curmi,P.A. (2008b). YB-1 promotes microtubule assembly in vitro through interaction with tubulin and microtubules. *BMC. Biochem.,* **9,** 23.

Chretien,D., Janosi,I., Taveau,J.C., and Flyvbjerg,H. (1999). Microtubule's conformational cap. *Cell Struct. Funct.,* **24,** 299-303.

Chuong,S.D., Good,A.G., Taylor,G.J., Freeman,M.C., Moorhead,G.B., and Muench,D.G. (2004). Large-scale identification of tubulin-binding proteins provides insight on subcellular trafficking, metabolic channeling, and signaling in plant cells. *Mol. Cell Proteomics.,* **3,** 970-983.

Conway,G., Wooley,J., Bibring,T., and LeStourgeon,W.M. (1988). Ribonucleoproteins package 700 nucleotides of pre-mRNA into a repeating array of regular particles. *Mol. Cell Biol.,* **8,** 2884-2895.

Cragg,G.M. (1998). Paclitaxel (Taxol): a success story with valuable lessons for natural product drug discovery and development. *Med. Res. Rev.,* **18,** 315-331.

Curmi,P.A., Andersen,S.S.L., Lachkar,S., Gavet,O., Karsenti,E., Knossow,M., and Sobel,A. (1997). The stathmin/tubulin interaction in vitro. *J. Biol. Chem.,* **272,** 25029-25036.

Curmi,P.A., Gavet,O., Charbaut,E., Ozon,S., Lachkar-Colmerauer,S., Manceau,V., Siavoshian,S., Maucuer,A., and Sobel,A. (1999). Stathmin and its phosphoprotein family: general properties, biochemical and functional interaction with tubulin. *Cell Struct. Funct.,* **24,** 345-357.

Davydova,E.K., Evdokimova,V.M., Ovchinnikov,L.P., and Hershey,J.W. (1997). Overexpression in COS cells of p50, the major core protein associated with mRNA, results in translation inhibition. *Nucleic Acids Res.,* **25,** 2911-2916.

de Mareuil,J., Carre,M., Barbier,P., Campbell,G.R., Lancelot,S., Opi,S., Esquieu,D., Watkins,J.D., Prevot,C., Braguer,D., Peyrot,V., and Loret,E.P. (2005). HIV-1 Tat protein enhances microtubule polymerization. *Retrovirology.,* **2,** 5.

Dehmelt,L. and Halpain,S. (2004). Actin and microtubules in neurite initiation: are MAPs the missing link? *J. Neurobiol.,* **58,** 18-33.

Delacourte,A. and Defossez,A. (1986). Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments. *J. Neurol. Sci.,* **76,** 173-186.

Deo,R.C., Bonanno,J.B., Sonenberg,N., and Burley,S.K. (1999). Recognition of polyadenylate RNA by the poly(A)-binding protein. *Cell,* **98,** 835-845.

Desai,A. and Mitchison,T.J. (1997). Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol,* **13,** 83-117.

Deschamps,S., Viel,A., Garrigos,M., Denis,H., and le Maire,M. (1992). mRNP4, a major mRNA-binding protein from Xenopus oocytes is identical to transcription factor FRG Y2. *J. Biol. Chem.,* **267,** 13799-13802.

Dhamodharan,R., Jordan,M.A., Thrower,D., Wilson,L., and Wadsworth,P. (1995). Vinblastine suppresses dynamics of individual microtubules in living interphase cells. *Mol. Biol. Cell,* **6,** 1215-1229.

Didier,D.K., Schiffenbauer,J., Woulfe,S.L., Zacheis,M., and Schwartz,B.D. (1988). Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. *Proc. Natl. Acad. Sci. U. S. A,* **85,** 7322-7326.

Drechsel,D.N., Hyman,A.A., Cobb,M.H., and Kirschner,M.W. (1992). Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol. Biol Cell,* **3,** 1141-1154.

Drewes,G., Ebneth,A., and Mandelkow,E.M. (1998). MAPs, MARKs and microtubule dynamics. *Trends Biochem Sci.,* **23,** 307-311.

Drewes,G., Ebneth,A., Preuss,U., Mandelkow,E.M., and Mandelkow,E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule- associated proteins and trigger microtubule disruption. *Cell,* **89,** 297-308.

Dreyfuss,G., Kim,V.N., and Kataoka,N. (2002b). Messenger-RNA-binding proteins and the messages they carry. *Nat. Rev. Mol. Cell Biol.,* **3,** 195-205.

Dreyfuss,G., Kim,V.N., and Kataoka,N. (2002a). Messenger-RNA-binding proteins and the messages they carry. *Nat. Rev. Mol. Cell Biol.,* **3,** 195-205.

Durso,N.A. and Cyr,R.J. (1994). A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1 alpha. *Plant Cell,* **6,** 893-905.

Erickson,H.P. and O'Brien,E.T. (1992). Microtubule dynamic instability and GTP hydrolysis. *Annu. Rev. Biophys. Biomol. Struct.,* **21,** 145-166.

Erickson,H.P. and Voter,W.A. (1976c). Polycation-induced assembly of purified tubulin. *Proc. Natl. Acad. Sci. U. S. A,* **73,** 2813-2817.

Erickson,H.P. and Voter,W.A. (1976a). Polycation-induced assembly of purified tubulin. *Proc. Natl. Acad. Sci. U. S. A,* **73,** 2813-2817.

Erickson,H.P. and Voter,W.A. (1976b). Polycation-induced assembly of purified tubulin. *Proc. Natl. Acad. Sci. U. S. A,* **73,** 2813-2817.

Evdokimova,V., Ruzanov,P., Anglesio,M.S., Sorokin,A.V., Ovchinnikov,L.P., Buckley,J., Triche,T.J., Sonenberg,N., and Sorensen,P.H. (2006). Akt-mediated YB-1 phosphorylation activates translation of silent mRNA species. *Mol. Cell Biol.,* **26,** 277-292.

Evdokimova,V., Ruzanov,P., Imataka,H., Raught,B., Svitkin,Y., Ovchinnikov,L.P., and Sonenberg,N. (2001). The major mRNA-associated protein YB-1 is a potent 5' cap-dependent mRNA stabilizer. *EMBO J.,* **20,** 5491-5502.

Evdokimova,V.M., Wei,C.L., Sitikov,A.S., Simonenko,P.N., Lazarev,O.A., Vasilenko,K.S., Ustinov,V.A., Hershey,J.W., and Ovchinnikov,L.P. (1995). The major protein of messenger ribonucleoprotein particles in somatic cells is a member of the Y-box binding transcription factor family. *J. Biol. Chem.,* **270,** 3186-3192.

Fan,J., Griffiths,A.D., Lockhart,A., Cross,R.A., and Amos,L.A. (1996). Microtubule minus ends can be labelled with a phage display antibody specific to alpha-tubulin. *J. Mol. Biol.,* **259,** 325-330.

Flyvbjerg,H., Jobs,E., and Leibler,S. (1996b). Kinetics of self-assembling microtubules: an "inverse problem" in biochemistry. *Proc. Natl. Acad. Sci. U. S. A,* **93,** 5975-5979.

Flyvbjerg,H., Jobs,E., and Leibler,S. (1996a). Kinetics of self-assembling microtubules: an "inverse problem" in biochemistry. *Proc. Natl. Acad. Sci. U. S. A,* **93,** 5975-5979.

Gard,D.L. and Kirschner,M.W. (1987a). A microtubule-associated protein from Xenopus eggs that specifically promotes assembly at the plus-end. *J. Cell Biol.,* **105,** 2203-2215.

Gard,D.L. and Kirschner,M.W. (1987b). Microtubule assembly in cytoplasmic extracts of Xenopus oocytes and eggs. *J. Cell Biol.,* **105,** 2191-2201.

Gaudreault,I., Guay,D., and Lebel,M. (2004). YB-1 promotes strand separation in vitro of duplex DNA containing either mispaired bases or cisplatin modifications, exhibits endonucleolytic activities and binds several DNA repair proteins. *Nucleic Acids Res.,* **32,** 316-327.

Gigant,B., Curmi,P.A., Martin-Barbey,C., Charbaut,E., Lachkar,S., Lebeau,L., Siavoshian,S., Sobel,A., and Knossow,M. (2000). The 4 A X-ray structure of a tubulin:stathmin-like domain complex. *Cell,* **102,** 809-816.

Gigant,B., Wang,C., Ravelli,R.B., Roussi,F., Steinmetz,M.O., Curmi,P.A., Sobel,A., and Knossow,M. (2005). Structural basis for the regulation of tubulin by vinblastine. *Nature,* **435,** 519-522.

Gilks,N., Kedersha,N., Ayodele,M., Shen,L., Stoecklin,G., Dember,L.M., and Anderson,P. (2004). Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol. Biol. Cell,* **15,** 5383-5398.

Goldstein,J., Pollitt,N.S., and Inouye,M. (1990). Major cold shock protein of Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A,* **87,** 283-287.

Gorgoni,B. and Gray,N.K. (2004). The roles of cytoplasmic poly(A)-binding proteins in regulating gene expression: a developmental perspective. *Brief. Funct. Genomic. Proteomic.,* **3,** 125-141.

Grabarek,Z. and Gergely,J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal. Biochem.,* **185,** 131-135.

Graumann,P.L. and Marahiel,M.A. (1998). A superfamily of proteins that contain the cold-shock domain. *Trends Biochem. Sci.,* **23,** 286-290.

Guillaud,L., Bosc,C., Fourest-Lieuvin,A., Denarier,E., Pirollet,F., Lafanechere,L., and Job,D. (1998). STOP proteins are responsible for the high degree of microtubule stabilization observed in neuronal cells. *J. Cell Biol.,* **142,** 167-179.

Halpain,S. and Dehmelt,L. (2006). The MAP1 family of microtubule-associated proteins. *Genome Biol.,* **7,** 224.

Hazan,J., Fonknechten,N., Mavel,D., Paternotte,C., Samson,D., Artiguenave,F., Davoine,C.S., Cruaud,C., Durr,A., Wincker,P., Brottier,P., Cattolico,L., Barbe,V., Burgunder,J.M., Prud'homme,J.F., Brice,A., Fontaine,B., Heilig,B., and Weissenbach,J. (1999). Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat. Genet.,* **23,** 296-303.

Heinlein,M., Padgett,H.S., Gens,J.S., Pickard,B.G., Casper,S.J., Epel,B.L., and Beachy,R.N. (1998). Changing patterns of localization of the tobacco mosaic virus movement protein and replicase to the endoplasmic reticulum and microtubules during infection. *Plant Cell,* **10,** 1107-1120.

Honore,S., Pasquier,E., and Braguer,D. (2005). Understanding microtubule dynamics for improved cancer therapy. *Cell Mol. Life Sci.,* **62,** 3039-3056.

Horn,G., Hofweber,R., Kremer,W., and Kalbitzer,H.R. (2007). Structure and function of bacterial cold shock proteins. *Cell Mol. Life Sci.,* **64,** 1457-1470.

Hugdahl,J.D., Bokros,C.L., and Morejohn,L.C. (1995). End-to-end annealing of plant microtubules by the p86 subunit of eukaryotic initiation factor-(iso)4F. *Plant Cell,* **7,** 2129-2138.

Hyman,A.A., Chretien,D., Arnal,I., and Wade,R.H. (1995). Structural changes accompanying GTP hydrolysis in microtubules: information from a slowly hydrolyzable analogue guanylyl-(alpha,beta)-methylene-diphosphonate. *J. Cell Biol.,* **128,** 117-125.

Hyman,A.A., Salser,S., Drechsel,D.N., Unwin,N., and Mitchison,T.J. (1992). Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP. *Mol Biol Cell,* **3,** 1155-1167.

Igarashi,K. and Kashiwagi,K. (2000). Polyamines: mysterious modulators of cellular functions. *Biochem. Biophys. Res. Commun.,* **271,** 559-564.

Ivanov,P.A., Chudinova,E.M., and Nadezhdina,E.S. (2003a). Disruption of microtubules inhibits cytoplasmic ribonucleoprotein stress granule formation. *Exp. Cell Res.,* **290,** 227-233.

Ivanov,P.A., Chudinova,E.M., and Nadezhdina,E.S. (2003b). Disruption of microtubules inhibits cytoplasmic ribonucleoprotein stress granule formation. *Exp. Cell Res.,* **290,** 227-233.

Izumi,H., Imamura,T., Nagatani,G., Ise,T., Murakami,T., Uramoto,H., Torigoe,T., Ishiguchi,H., Yoshida,Y., Nomoto,M., Okamoto,T., Uchiumi,T., Kuwano,M., Funa,K., and Kohno,K. (2001). Y box-binding protein-1 binds preferentially to single-stranded nucleic acids and exhibits 3'-->5' exonuclease activity. *Nucleic Acids Res.,* **29,** 1200-1207.

Janosi,I.M., Chretien,D., and Flyvbjerg,H. (2002). Structural microtubule cap: stability, catastrophe, rescue, and third state. *Biophys. J.,* **83,** 1317-1330.

Janz,M., Jurchott,K., Karawajev L., and Royer H.-D. (2000). Y-box factor YB-1 is associated with the centrosome during mitosis. *Gene Funct. Dis.,* **1,** 57-59.

Jiang,W., Hou,Y., and Inouye,M. (1997). CspA, the major cold-shock protein of Escherichia coli, is an RNA chaperone. *J. Biol. Chem.,* **272,** 196-202.

Job,D., Valiron,O., and Oakley,B. (2003). Microtubule nucleation. *Curr Opin Cell Biol,* **15,** 111-117.

Jordan,M.A., Thrower,D., and Wilson,L. (1992). Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. *J Cell Sci.,* **102,** 401-416.

Jordan,M.A., Toso,R.J., Thrower,D., and Wilson,L. (1993). Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl. Acad. Sci. U. S. A,* **90,** 9552-9556.

Jordan,M.A. and Wilson,L. (1998). Microtubules and actin filaments: dynamic targets for cancer chemotherapy. *Curr. Opin. Cell Biol.,* **10,** 123-130.

Jordan,M.A. and Wilson,L. (2004). Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer,* **4,** 253-265.

Jorgensen,R.A., Atkinson,R.G., Forster,R.L., and Lucas,W.J. (1998). An RNA-based information superhighway in plants. *Science,* **279,** 1486-1487.

Jourdain,L., Curmi,P., Sobel,A., Pantaloni,D., and Carlier,M.F. (1997). Stathmin: A tubulin-sequestering protein which forms a ternary T2S complex with two tubulin molecules. *Biochemistry,* **36,** 10817-10821.

Kar,S., Fan,J., Smith,M.J., Goedert,M., and Amos,L.A. (2003). Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. *EMBO J.,* **22,** 70-77.

Kedersha,N. and Anderson,P. (2007). Mammalian stress granules and processing bodies. *Methods Enzymol.,* **431,** 61-81.

Kedersha,N., Stoecklin,G., Ayodele,M., Yacono,P., Lykke-Andersen,J., Fritzler,M.J., Scheuner,D., Kaufman,R.J., Golan,D.E., and Anderson,P. (2005). Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J. Cell Biol.,* **169,** 871-884.

Kick,D., Barrett,P., Cummings,A., and Sommerville,J. (1987b). Phosphorylation of a 60 kDa polypeptide from Xenopus oocytes blocks messenger RNA translation. *Nucleic Acids Res.,* **15,** 4099-4109.

Kick,D., Barrett,P., Cummings,A., and Sommerville,J. (1987a). Phosphorylation of a 60 kDa polypeptide from Xenopus oocytes blocks messenger RNA translation. *Nucleic Acids Res.,* **15,** 4099-4109.

Kikkawa,M. (2008). The role of microtubules in processive kinesin movement. *Trends Cell Biol.,* **18,** 128-135.

King,S.J. and Schroer,T.A. (2000). Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat. Cell Biol.,* **2,** 20-24.

Kinoshita,K., Arnal,I., Desai,A., Drechsel,D.N., and Hyman,A.A. (2001). Reconstitution of physiological microtubule dynamics using purified components. *Science,* **294,** 1340-1343.

Kinoshita,K., Habermann,B., and Hyman,A.A. (2002). XMAP215: a key component of the dynamic microtubule cytoskeleton. *Trends Cell Biol,* **12,** 267-273.

Kirschner,M.W. (1980). Implications of treadmilling for the stability and polarity of actin and tubulin polymers in vivo. *J. Cell Biol.,* **86,** 330-334.

Kiselyova,O.I., Yaminsky,I.V., Karger,E.M., Frolova,O.Y., Dorokhov,Y.L., and Atabekov,J.G. (2001). Visualization by atomic force microscopy of tobacco mosaic virus movement protein-RNA complexes formed in vitro. *J. Gen. Virol.,* **82,** 1503-1508.

Kline-Smith,S.L. and Walczak,C.E. (2004). Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics. *Mol. Cell,* **15,** 317-327.

Kloks,C.P., Spronk,C.A., Lasonder,E., Hoffmann,A., Vuister,G.W., Grzesiek,S., and Hilbers,C.W. (2002). The solution structure and DNA-binding properties of the cold-shock domain of the human Y-box protein YB-1. *J. Mol. Biol.,* **316,** 317-326.

Kozlov,G., Trempe,J.F., Khaleghpour,K., Kahvejian,A., Ekiel,I., and Gehring,K. (2001). Structure and function of the C-terminal PABC domain of human poly(A)-binding protein. *Proc. Natl. Acad. Sci. U. S. A,* **98,** 4409-4413.

Krauhs,E., Little,M., Kempf,T., Hofer-Warbinek,R., Ade,W., and Ponstingl,H. (1981). Complete amino acid sequence of beta-tubulin from porcine brain. *Proc. Natl. Acad. Sci. U. S. A,* **78,** 4156-4160.

Kuriyama,R., Savereide,P., Lefebvre,P., and Dasgupta,S. (1990). The predicted amino acid sequence of a centrosphere protein in dividing sea urchin eggs is similar to elongation factor (EF-1 alpha). *J. Cell Sci.,* **95 (Pt 2),** 231-236.

Ladomery,M. and Sommerville,J. (1994a). Binding of Y-box proteins to RNA: involvement of different protein domains. *Nucleic Acids Res.,* **22,** 5582-5589.

Ladomery,M. and Sommerville,J. (1994b). Binding of Y-box proteins to RNA: involvement of different protein domains. *Nucleic Acids Res.,* **22,** 5582-5589.

Ladomery,M., Wade,E., and Sommerville,J. (1997). Xp54, the Xenopus homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. *Nucleic Acids Res.,* **25,** 965-973.

Landsman,D. (1992). RNP-1, an RNA-binding motif is conserved in the DNA-binding cold shock domain. *Nucleic Acids Res.,* **20,** 2861-2864.

Leguy,R., Melki,R., Pantaloni,D., and Carlier,M.F. (2000). Monomeric gamma -tubulin nucleates microtubules. *J Biol Chem,* **275,** 21975-21980.

Luders,J. and Stearns,T. (2007). Microtubule-organizing centres: a re-evaluation. *Nat. Rev. Mol. Cell Biol.,* **8,** 161-167.

Maccioni,R.B., Rivas,C.I., and Vera,J.C. (1988). Differential interaction of synthetic peptides from the carboxyl-terminal regulatory domain of tubulin with microtubule-associated proteins. *EMBO J.,* **7,** 1957-1963.

Mahoney,N.M., Goshima,G., Douglass,A.D., and Vale,R.D. (2006). Making microtubules and mitotic spindles in cells without functional centrosomes. *Curr. Biol.,* **16,** 564-569.

Mallik,R. and Gross,S.P. (2004). Molecular motors: strategies to get along. *Curr. Biol.,* **14,** R971-R982.

Mandelkow,E. and Mandelkow,E.M. (1995). Microtubules and microtubule-associated proteins. *Curr. Opin. Cell Biol,* **7,** 72-81.

Mandelkow,E.M., Mandelkow,E., and Milligan,R.A. (1991). Microtubule dynamics and microtubule caps: a time-resolved cryo-electron microscopy study. *J Cell Biol,* **114,** 977-991.

Mangus,D.A., Evans,M.C., and Jacobson,A. (2003). Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol.,* **4,** 223.

Maniatis,T. and Reed,R. (2002). An extensive network of coupling among gene expression machines. *Nature,* **416,** 499-506.

Manna,T., Thrower,D., Miller,H.P., Curmi,P., and Wilson,L. (2006). Stathmin strongly increases the minus end catastrophe frequency and induces rapid treadmilling of bovine brain microtubules at steady state in vitro. *J. Biol. Chem.,* **281,** 2071-2078.

Marchesi,V.T. and Ngo,N. (1993). In vitro assembly of multiprotein complexes containing alpha, beta, and gamma tubulin, heat shock protein HSP70, and elongation factor 1 alpha. *Proc. Natl. Acad. Sci. U. S. A,* **90,** 3028-3032.

Marcum,J.M. and Borisy,G.G. (1978). Characterization of microtubule protein oligomers by analytical ultracentrifugation. *J. Biol. Chem.,* **253,** 2825-2833.

Matsumoto,K., Tanaka,K.J., Aoki,K., Sameshima,M., and Tsujimoto,M. (2003). Visualization of the reconstituted FRGY2-mRNA complexes by electron microscopy. *Biochem. Biophys. Res. Commun.,* **306,** 53-58.

Mechulam,A., Chernov,K.G., Mucher,E., Hamon,L., Curmi,P.A., and Pastre,D. (2009). Polyamine sharing between tubulin dimers favours microtubule nucleation and elongation via facilitated diffusion. *PLoS. Comput. Biol.,* **5,** e1000255.

Mejillano,M.R. and Himes,R.H. (1991b). Assembly properties of tubulin after carboxyl group modification. *J. Biol. Chem.,* **266,** 657-664.

Mejillano,M.R. and Himes,R.H. (1991a). Assembly properties of tubulin after carboxyl group modification. *J. Biol. Chem.,* **266,** 657-664.

Melki,R., Fievez,S., and Carlier,M.F. (1996). Continuous monitoring of Pi release following nucleotide hydrolysis in actin or tubulin assembly using 2-amino-6-mercapto-7-methylpurine ribonucleoside and purine-nucleoside phosphorylase as an enzyme-linked assay. *Biochemistry,* **35,** 12038-12045.

Melki,R., Kerjan,P., Waller,J.P., Carlier,M.F., and Pantaloni,D. (1991). Interaction of microtubule-associated proteins with microtubules: yeast lysyl- and valyl-tRNA synthetases and tau 218-235 synthetic peptide as model systems. *Biochemistry,* **30,** 11536-11545.

Minich,W.B., Maidebura,I.P., and Ovchinnikov,L.P. (1993). Purification and characterization of the major 50-kDa repressor protein from cytoplasmic mRNP of rabbit reticulocytes. *Eur. J. Biochem.,* **212,** 633-638.

Mitchison,T.J. (1993). Localization of an exchangeable GTP binding site at the plus end of microtubules. *Science,* **261,** 1044-1047.

Mitchison,T.J. and Kirschner,M. (1984). Dynamic instability of microtubule growth. *Nature,* **312,** 237-242.

Mithieux,G., Alquier,C., Roux,B., and Rousset,B. (1984b). Interaction of tubulin with chromatin proteins. H1 and core histones. *J. Biol. Chem.,* **259,** 15523-15531.

Mithieux,G., Alquier,C., Roux,B., and Rousset,B. (1984a). Interaction of tubulin with chromatin proteins. H1 and core histones. *J. Biol. Chem.,* **259,** 15523-15531.

Mithieux,G., Alquier,C., Roux,B., and Rousset,B. (1984c). Interaction of tubulin with chromatin proteins. H1 and core histones. *J. Biol. Chem.,* **259,** 15523-15531.

Miwa,A., Higuchi,T., and Kobayashi,S. (2006). Expression and polysome association of YB-1 in various tissues at different stages in the lifespan of mice. *Biochim. Biophys. Acta,* **1760,** 1675-1681.

Moreland,J.L., Gramada,A., Buzko,O.V., Zhang,Q., and Bourne,P.E. (2005). The Molecular Biology Toolkit (MBT): a modular platform for developing molecular visualization applications. *BMC. Bioinformatics.,* **6,** 21.

Muller-Reichert,T., Chretien,D., Severin,F., and Hyman,A.A. (1998). Structural changes at microtubule ends accompanying GTP hydrolysis: information from a slowly hydrolyzable analogue of GTP, guanylyl (alpha,beta)methylenediphosphonate. *Proc. Natl. Acad. Sci. U. S. A,* **95,** 3661-3666.

Murphy,D.B. and Borisy,G.G. (1975). Association of high-molecular-weight proteins with microtubules and their role in microtubule assembly in vitro. *Proc. Natl. Acad. Sci. U. S. A,* **72,** 2696-2700.

Murzin,A.G. (1993). OB(oligonucleotide/oligosaccharide binding)-fold: common structural and functional solution for non-homologous sequences. *EMBO J.,* **12,** 861-867.

Nakamura,A., Amikura,R., Hanyu,K., and Kobayashi,S. (2001). Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. *Development,* **128,** 3233-3242.

Nashchekin,D., Zhao,J., Visa,N., and Daneholt,B. (2006b). A novel Ded1-like RNA helicase interacts with the Y-box protein ctYB-1 in nuclear mRNP particles and in polysomes. *J. Biol. Chem.,* **281,** 14263-14272.

Nashchekin,D., Zhao,J., Visa,N., and Daneholt,B. (2006a). A novel Ded1-like RNA helicase interacts with the Y-box protein ctYB-1 in nuclear mRNP particles and in polysomes. *J. Biol. Chem.,* **281,** 14263-14272.

Nekrasov,M.P., Ivshina,M.P., Chernov,K.G., Kovrigina,E.A., Evdokimova,V.M., Thomas,A.A., Hershey,J.W., and Ovchinnikov,L.P. (2003). The mRNA-binding protein YB-1 (p50) prevents association of the eukaryotic initiation factor eIF4G with mRNA and inhibits protein synthesis at the initiation stage. *J Biol Chem,* **278,** 13936-13943.

Nogales,E., Medrano,F.J., Diakun,G.P., Mant,G.R., Towns-Andrews,E., and Bordas,J. (1995a). The effect of temperature on the structure of vinblastine-induced polymers of purified tubulin: detection of a reversible conformational change. *J. Mol. Biol.,* **254,** 416-430.

Nogales,E., Wolf,S.G., and Downing,K.H. (1998). Structure of the alpha beta tubulin dimer by electron crystallography. *Nature,* **391,** 199-203.

Nogales,E., Wolf,S.G., Khan,I.A., Luduena,R.F., and Downing,K.H. (1995b). Structure of tubulin at 6.5 A and location of the taxol-binding site. *Nature,* **375,** 424-427.

Odde,D.J. (1997b). Estimation of the diffusion-limited rate of microtubule assembly. *Biophys. J.,* **73,** 88-96.

Odde,D.J. (1997c). Estimation of the diffusion-limited rate of microtubule assembly. *Biophys. J.,* **73,** 88-96.

Odde,D.J. (1997a). Estimation of the diffusion-limited rate of microtubule assembly. *Biophys. J.,* **73,** 88-96.

Ohta,K., Toriyama,M., Miyazaki,M., Murofushi,H., Hosoda,S., Endo,S., and Sakai,H. (1990). The mitotic apparatus-associated 51-kDa protein from sea urchin eggs is a GTP-binding protein and is immunologically related to yeast polypeptide elongation factor 1 alpha. *J. Biol. Chem.,* **265,** 3240-3247.

Okamoto,T., Izumi,H., Imamura,T., Takano,H., Ise,T., Uchiumi,T., Kuwano,M., and Kohno,K. (2000). Direct interaction of p53 with the Y-box binding protein, YB-1: a mechanism for regulation of human gene expression. *Oncogene,* **19,** 6194-6202.

Oredsson,S.M. (2003). Polyamine dependence of normal cell-cycle progression. *Biochem. Soc. Trans.,* **31,** 366-370.

Otter,A., Scott,P.G., Maccioni,R.B., and Kotovych,G. (1991). The solution conformation of tubulin-beta(422-434)-NH2 and its Nac-DATADEQG-NH2 fragment based on NMR. *Biopolymers,* **31,** 449-458.

Ovchinnikov,L.P., Avanesov,A.T., Seriakova,T.A., Alzhanova,A.T., and Radzhabov,H.M. (1978a). A comparison of the RNA-binding proteins with the proteins of polyribosomal messenger ribonucleoproteins in rabbit reticulocytes. *Eur. J. Biochem.,* **90,** 527-535.

Ovchinnikov,L.P., Seriakova,T.A., Avanesov,A.T., Alzhanova,A.T., Radzhabov,H.M., and Spirin,A.S. (1978b). RNA-binding proteins of rabbit reticulocytes. Isolation and electrophoretic characteristics. *Eur. J. Biochem.,* **90,** 517-525.

Ovchinnikov,L.P., Skabkin,M.A., Ruzanov,P.V., and Evdokimova,V.M. (2001). [Major mRNP proteins in the structural organization and function of mRNA in eukaryotic cells]. *Mol. Biol. (Mosk),* **35,** 548-558.

Ozer,J., Faber,M., Chalkley,R., and Sealy,L. (1990). Isolation and characterization of a cDNA clone for the CCAAT transcription factor EFIA reveals a novel structural motif. *J. Biol. Chem.,* **265,** 22143-22152.

Palacios,I.M. and St Johnston,D. (2001). Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu. Rev. Cell Dev. Biol.,* **17,** 569-614.

Panda,D., Goode,B.L., Feinstein,S.C., and Wilson,L. (1995b). Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. *Biochemistry,* **34,** 11117-11127.

Panda,D., Goode,B.L., Feinstein,S.C., and Wilson,L. (1995a). Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. *Biochemistry,* **34,** 11117-11127.

Pastre,D., Pietrement,O., Fusil,S., Landousy,F., Jeusset,J., David,M.O., Hamon,L., Le Cam,E., and Zozime,A. (2003). Adsorption of DNA to mica mediated by divalent counterions: a theoretical and experimental study. *Biophys. J.,* **85,** 2507-2518.

Pegg,A.E. (2006). Regulation of ornithine decarboxylase. *J. Biol. Chem.,* **281,** 14529-14532.

Phadtare,S., Inouye,M., and Severinov,K. (2002). The nucleic acid melting activity of Escherichia coli CspE is critical for transcription antitermination and cold acclimation of cells. *J. Biol. Chem.,* **277,** 7239-7245.

Phizicky,E.M. and Fields,S. (1995). Protein-protein interactions: methods for detection and analysis. *Microbiol. Rev.,* **59,** 94-123.

Pohjanpelto,P., Virtanen,I., and Holtta,E. (1981b). Polyamine starvation causes disappearance of actin filaments and microtubules in polyamine-auxotrophic CHO cells. *Nature,* **293,** 475-477.

Pohjanpelto,P., Virtanen,I., and Holtta,E. (1981a). Polyamine starvation causes disappearance of actin filaments and microtubules in polyamine-auxotrophic CHO cells. *Nature,* **293,** 475-477.

Ponstingl,H., Krauhs,E., Little,M., and Kempf,T. (1981). Complete amino acid sequence of alpha-tubulin from porcine brain. *Proc. Natl. Acad. Sci. U. S. A,* **78,** 2757-2761.

Preobrazhensky,A.A. and Spirin,A.S. (1978). Informosomes and their protein components: the present state of knowledge. *Prog. Nucleic Acid Res. Mol. Biol.,* **21,** 1-38.

Quarmby,L. (2000). Cellular Samurai: katanin and the severing of microtubules. *J. Cell Sci.,* **113 (Pt 16),** 2821-2827.

Ravelli,R.B.G., Gigant,B., Curmi,P.A., Jourdain,I., Lachkar,S., Sobel,A., and Knossow,M. (2004). Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature,* **428,** 198-202.

Raynaud-Messina,B. and Merdes,A. (2007). [gamma]-tubulin complexes and microtubule organization. *Curr. Op. Cell Biol.,* **19,** 24-30.

Rezania,V., Azarenko,O., Jordan,M.A., Bolterauer,H., Luduena,R.F., Huzil,J.T., and Tuszynski,J.A. (2008). Microtubule assembly of isotypically purified tubulin and its mixtures. *Biophys. J.,* **95,** 1993-2008.

Rice,S., Lin,A.W., Safer,D., Hart,C.L., Naber,N., Carragher,B.O., Cain,S.M., Pechatnikova,E., Wilson-Kubalek,E.M., Whittaker,M., Pate,E., Cooke,R., Taylor,E.W., Milligan,R.A., and Vale,R.D. (1999). A structural change in the kinesin motor protein that drives motility. *Nature,* **402,** 778-784.

Rost,B., Yachdav,G., and Liu,J. (2004). The PredictProtein server. *Nucleic Acids Res.,* **32,** W321-W326.

Ruzanov,P.V., Evdokimova,V.M., Korneeva,N.L., Hershey,J.W., and Ovchinnikov,L.P. (1999). Interaction of the universal mRNA-binding protein, p50, with actin: a possible link between mRNA and microfilaments. *J. Cell Sci.,* **112 (Pt 20),** 3487-3496.

Sackett,D.L., Bhattacharyya,B., and Wolff,J. (1985a). Tubulin subunit carboxyl termini determine polymerization efficiency. *J. Biol. Chem.,* **260,** 43-45.

Sackett,D.L., Bhattacharyya,B., and Wolff,J. (1985b). Tubulin subunit carboxyl termini determine polymerization efficiency. *J. Biol. Chem.,* **260,** 43-45.

Safak,M., Gallia,G.L., and Khalili,K. (1999). Reciprocal interaction between two cellular proteins, Puralpha and YB-1, modulates transcriptional activity of JCVCY in glial cells. *Mol. Cell Biol.,* **19,** 2712-2723.

Sakura,H., Maekawa,T., Imamoto,F., Yasuda,K., and Ishii,S. (1988). Two human genes isolated by a novel method encode DNA-binding proteins containing a common region of homology. *Gene,* **73,** 499-507.

Salinas,S., Carazo-Salas,R.E., Proukakis,C., Schiavo,G., and Warner,T.T. (2007). Spastin and microtubules: Functions in health and disease. *J. Neurosci. Res.,* **85,** 2778-2782.

Salmon,E.D., Saxton,W.M., Leslie,R.J., Karow,M.L., and McIntosh,J.R. (1984). Diffusion coefficient of fluorescein-labeled tubulin in the cytoplasm of embryonic cells of a sea urchin: video image analysis of fluorescence redistribution after photobleaching. *J. Cell Biol.,* **99,** 2157-2164.

Samarina,O.P., Lukanidin,E.M., Molnar,J., and Georgiev,G.P. (1968). Structural organization of nuclear complexes containing DNA-like RNA. *J. Mol. Biol.,* **33,** 251-263.

Scheele,R.B. and Borisy,G.G. (1978). Electron microscopy of metal-shadowed and negatively stained microtubule protein. Structure of the 30 S oligomer. *J. Biol. Chem.,* **253,** 2846-2851.

Schiff,P.B., Fant,J., and Horwitz,S.B. (1979). Promotion of microtubule assembly in vitro by taxol. *Nature,* **277,** 665-667.

Schindelin,H., Jiang,W., Inouye,M., and Heinemann,U. (1994). Crystal structure of CspA, the major cold shock protein of Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A,* **91,** 5119-5123.

Schindler,T., Graumann,P.L., Perl,D., Ma,S., Schmid,F.X., and Marahiel,M.A. (1999). The family of cold shock proteins of Bacillus subtilis. Stability and dynamics in vitro and in vivo. *J. Biol. Chem.,* **274,** 3407-3413.

Schindler,T., Perl,D., Graumann,P., Sieber,V., Marahiel,M.A., and Schmid,F.X. (1998). Surface-exposed phenylalanines in the RNP1/RNP2 motif stabilize the cold-shock protein CspB from Bacillus subtilis. *Proteins,* **30,** 401-406.

Schlieper,D., Oliva,M.A., Andreu,J.M., and Lowe,J. (2005). Structure of bacterial tubulin BtubA/B: evidence for horizontal gene transfer. *Proc. Natl. Acad. Sci. U. S. A,* **102,** 9170-9175.

Schneider,R.J. and Mohr,I. (2003). Translation initiation and viral tricks. *Trends Biochem. Sci.,* **28,** 130-136.

Schnuchel,A., Wiltscheck,R., Czisch,M., Herrler,M., Willimsky,G., Graumann,P., Marahiel,M.A., and Holak,T.A. (1993). Structure in solution of the major cold-shock protein from Bacillus subtilis. *Nature,* **364,** 169-171.

Schroder,K., Graumann,P., Schnuchel,A., Holak,T.A., and Marahiel,M.A. (1995). Mutational analysis of the putative nucleic acid-binding surface of the cold-shock domain, CspB, revealed an essential role of aromatic and basic residues in binding of single-stranded DNA containing the Y-box motif. *Mol. Microbiol.,* **16,** 699-708.

Sengupta,D.N., Berkhout,B., Gatignol,A., Zhou,A.M., and Silverman,R.H. (1990). Direct evidence for translational regulation by leader RNA and Tat protein of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. U. S. A,* **87,** 7492-7496.

Shiina,N., Gotoh,Y., Kubomura,N., Iwamatsu,A., and Nishida,E. (1994). Microtubule severing by elongation factor 1 alpha. *Science,* **266,** 282-285.

Shlyakhtenko,L.S., Potaman,V.N., Sinden,R.R., and Lyubchenko,Y.L. (1998). Structure and dynamics of supercoil-stabilized DNA cruciforms. *J. Mol. Biol.,* **280,** 61-72.

Shnyreva,M., Schullery,D.S., Suzuki,H., Higaki,Y., and Bomsztyk,K. (2000). Interaction of two multifunctional proteins. Heterogeneous nuclear ribonucleoprotein K and Y-box-binding protein. *J. Biol. Chem.,* **275,** 15498-15503.

Skabkin,M.A., Kiselyova,O.I., Chernov,K.G., Sorokin,A.V., Dubrovin,E.V., Yaminsky,I.V., Vasiliev,V.D., and Ovchinnikov,L.P. (2004). Structural organization of mRNA complexes with major core mRNP protein YB-1. *Nucleic Acids Res.,* **32,** 5621-5635.

Skabkina,O.V., Lyabin,D.N., Skabkin,M.A., and Ovchinnikov,L.P. (2005). YB-1 autoregulates translation of its own mRNA at or prior to the step of 40S ribosomal subunit joining. *Mol. Cell Biol,* **25,** 3317-3323.

Sloboda,R.D., Dentler,W.L., and Rosenbaum,J.L. (1976). Microtubule-associated proteins and the stimulation of tubulin assembly in vitro. *Biochemistry,* **15,** 4497-4505.

Sloboda,R.D., Rudolph,S.A., Rosenbaum,J.L., and Greengard,P. (1975). Cyclic AMP-dependent endogenous phosphorylation of a microtubule-associated protein. *Proc. Natl. Acad. Sci. U. S. A,* **72,** 177-181.

Sobel,A. (1991). Stathmin: a relay phosphoprotein for multiple signal transduction ? *Trends Biochem. Sci.,* **16,** 301-305.

Soop,T., Nashchekin,D., Zhao,J., Sun,X., Alzhanova-Ericsson,A.T., Bjorkroth,B., Ovchinnikov,L., and Daneholt,B. (2003). A p50-like Y-box protein with a putative translational role becomes associated with pre-mRNA concomitant with transcription. *J. Cell Sci.,* **116,** 1493-1503.

Sorokin,A.V., Selyutina,A.A., Skabkin,M.A., Guryanov,S.G., Nazimov,I.V., Richard,C., Th'ng,J., Yau,J., Sorensen,P.H., Ovchinnikov,L.P., and Evdokimova,V. (2005). Proteasome-mediated cleavage of the Y-box-binding protein 1 is linked to DNA-damage stress response. *EMBO J.,* **24,** 3602-3612.

Spirin,A.S. (1978). Eukaryotic messenger RNA and informosomes. Omnia mea mecum porto. *FEBS Lett.,* **88,** 15-17.

Spirin,A.S. and Nemer,M. (1965). Messenger RNA in early sea-urchin embryos: cytoplasmic particles. *Science,* **150,** 214-217.

Stewart,R.J., Farrell,K.W., and Wilson,L. (1990). Role of GTP hydrolysis in microtubule polymerization: evidence for a coupled hydrolysis mechanism. *Biochemistry,* **29,** 6489-6498.

Sutherland,B.W., Kucab,J., Wu,J., Lee,C., Cheang,M.C., Yorida,E., Turbin,D., Dedhar,S., Nelson,C., Pollak,M., Leighton,G.H., Miller,K., Badve,S., Huntsman,D., Blake-Gilks,C., Chen,M., Pallen,C.J., and Dunn,S.E. (2005). Akt phosphorylates the Y-box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage-independent growth of breast cancer cells. *Oncogene,* **24,** 4281-4292.

Tafuri,S.R. and Wolffe,A.P. (1990). Xenopus Y-box transcription factors: molecular cloning, functional analysis and developmental regulation. *Proc. Natl. Acad. Sci. U. S. A,* **87,** 9028-9032.

Tafuri,S.R. and Wolffe,A.P. (1992). DNA binding, multimerization, and transcription stimulation by the Xenopus Y box proteins in vitro. *New Biol.,* **4,** 349-359.

Takanari,H., Morita,J., Yamanaka,H., Yada,K., Takahashi,A., and Izutsu,K. (1994). Effects of acidic pH on the formation of vinblastine-induced paracrystals in Chinese hamster ovary cells. *Biol. Cell,* **82,** 51-57.

Taupin,J.L., Tian,Q., Kedersha,N., Robertson,M., and Anderson,P. (1995). The RNA-binding protein TIAR is translocated from the nucleus to the cytoplasm during Fas-mediated apoptotic cell death. *Proc. Natl. Acad. Sci. U. S. A,* **92,** 1629-1633.

Teng,J., Takei,Y., Harada,A., Nakata,T., Chen,J., and Hirokawa,N. (2001). Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. *J. Cell Biol.,* **155,** 65-76.

Toso,R.J., Jordan,M.A., Farrell,K.W., Matsumoto,B., and Wilson,L. (1993). Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. *Biochemistry,* **32,** 1285-1293.

Toueille,M., Saint-Jean,B., Castroviejo,M., and Benedetto,J.P. (2007). The elongation factor 1A: a novel regulator in the DNA replication/repair protein network in wheat cells? *Plant Physiol Biochem.,* **45,** 113-118.

Ustinov,V.A., Skabkin,M.A., Nashchekin,D.V., Evdokimova,V.M., and Ovchinnikov,L.P. (1996). [Major p50 protein of the somatic cell cytoplasmic mRNP: expression in Escherichia coli, isolation, and some properties of the recombinant protein]. *Biokhimiia.,* **61,** 559-566.

Vallee,R.B. and Borisy,G.G. (1978). The non-tubulin component of microtubule protein oligomers. Effect on self-association and hydrodynamic properties. *J. Biol. Chem.,* **253,** 2834-2845.

Vater,W., Fritzsche,W., Schaper,A., Bohm,K.J., Unger,E., and Jovin,T.M. (1995). Scanning force microscopy of microtubules and polymorphic tubulin assemblies in air and in liquid. *J. Cell Sci.,* **108 (Pt 3),** 1063-1069.

Vivanco,I. and Sawyers,C.L. (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat. Rev. Cancer,* **2,** 489-501.

Voter,W.A. and Erickson,H.P. (1984a). The kinetics of microtubule assembly. Evidence for a two-stage nucleation mechanism. *J. Biol. Chem.,* **259,** 10430-10438.

Voter,W.A. and Erickson,H.P. (1984b). The kinetics of microtubule assembly. Evidence for a two-stage nucleation mechanism. *J. Biol. Chem.,* **259,** 10430-10438.

Walczak,C.E. (2000). Microtubule dynamics and tubulin interacting proteins. *Curr. Opin. Cell Biol,* **12,** 52-56.

Walczak,C.E., Mitchison,T.J., and Desai,A. (1996). XKCM1: a xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell,* **84,** 37-47.

Walker,R.A., O'Brien,E.T., Pryer,N.K., Soboeiro,M.F., Voter,W.A., Erickson,H.P., and Salmon,E.D. (1988c). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.,* **107,** 1437-1448.

Walker,R.A., O'Brien,E.T., Pryer,N.K., Soboeiro,M.F., Voter,W.A., Erickson,H.P., and Salmon,E.D. (1988a). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.,* **107,** 1437-1448.

Walker,R.A., O'Brien,E.T., Pryer,N.K., Soboeiro,M.F., Voter,W.A., Erickson,H.P., and Salmon,E.D. (1988b). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.,* **107,** 1437-1448.

Walker,R.A., Pryer,N.K., and Salmon,E.D. (1991). Dilution of individual microtubules observed in real time in vitro: evidence that cap size is small and independent of elongation rate. *J. Cell Biol.,* **114,** 73-81.

Waterman-Storer,C.M. and Salmon,E.D. (1997). Microtubule dynamics: treadmilling comes around again. *Curr Biol,* **7,** R369-R372.

Witman,G.B., Cleveland,D.W., Weingarten,M.D., and Kirschner,M.W. (1976). Tubulin requires tau for growth onto microtubule initiating sites. *Proc. Natl. Acad. Sci. U. S. A,* **73,** 4070-4074.

Wolff,J. (1998a). Promotion of microtubule assembly by oligocations: cooperativity between charged groups. *Biochemistry,* **37,** 10722-10729.

Wolff,J. (1998b). Promotion of microtubule assembly by oligocations: cooperativity between charged groups. *Biochemistry,* **37,** 10722-10729.

Wolff,J., Knipling,L., and Sackett,D.L. (1996). Charge-shielding and the "paradoxical" stimulation of tubulin polymerization by guanidine hydrochloride. *Biochemistry,* **35,** 5910-5920.

Wolffe,A.P., Tafuri,S., Ranjan,M., and Familari,M. (1992). The Y-box factors: a family of nucleic acid binding proteins conserved from Escherichia coli to man. *New Biol.,* **4,** 290-298.

Wu,J., Stradford,A., Astanehe,A., and Dunn S. (2007). YB-1 is a transcription/translation factor that orchestrates the oncogenome by hardwiring signal transduction to gene expression. *Translational Oncogenomics,* **2,** 49-65.

Yu,J., Hecht,N.B., and Schultz,R.M. (2001). Expression of MSY2 in mouse oocytes and preimplantation embryos. *Biol. Reprod.,* **65,** 1260-1270.

Yu,J., Hecht,N.B., and Schultz,R.M. (2002). RNA-binding properties and translation repression in vitro by germ cell-specific MSY2 protein. *Biol. Reprod.,* **67,** 1093-1098.

Zhao,H. and Sokhansanj,B.A. (2007). Integrated modeling methodology for microtubule dynamics and Taxol kinetics with experimentally identifiable parameters. *Comput. Methods Programs Biomed.,* **88,** 18-25.

Zheng,Y., Wong,M.L., Alberts,B., and Mitchison,T. (1995). Nucleation of microtubule assembly by a gamma-tubulin-containing ring complex. *Nature,* **378,** 578-583.

H. RESUME

YB-1 est un régulateur important de l expression des gènes dans les cellules eucaryotes. En plus de son rôle dans la transcription, YB-1 joue un rôle clé dans la traduction et la stabilisation des ARN messagers. Nous avons identifié plusieurs nouveaux partenaires de la protéine YB-1 par chromatographie d affinité à partir de différents extraits tissulaires. Parmi ces partenaires, nous avons démontré que YB-1 interagit avec la tubuline et les microtubules et stimule fortement l'assemblage des microtubules *in vitro*. Les microtubules assemblés en présence de YB-1 ont une ultrastructure normale, et les données montrent que YB-1 recouvre probablement la surface extérieure des microtubules. De la même façon YB-1 stimule aussi l'assemblage de la tubuline-MAP qui est plus proche des complexes protéiques qui existent dans la cellule, et de la tubuline clivée par subtilisine ce qui suggère que son interaction avec la tubuline ne relève pas seulement d effets électrostatiques. Nous avons enfin découvert que la tubuline interfère avec la formation des complexes ARNm:YB-1. Ces résultats suggèrent que YB-1 peut réguler l'assemblage des microtubules *in vivo* et que son interaction avec la tubuline peut contribuer à la régulation de la traduction des ARN messagers. En effet, *in vivo,* la traduction des mRNPs dépend de l état de saturation de l ARN messager par YB-1. Nous avons montré ici que lorsque le rapport YB-1:ARNm est faible, les complexes mRNPs possèdent des structures non-compactes, alors que les mRNPs saturés sont compacts. Ce changement structural est observé de façon parallèle à l'inhibition de la traduction des ARN messagers lorsqu ils passent des polysomes (*traduits*) aux mRNPs libres (*non traduits*). De façon intéressante, nous avons découvert que les mRNPs saturés se lient aux microtubules via des interactions protéine:protéine et ont tendance à former des agrégats sur la surface des microtubules. Cette dernière propriété pourrait contribuer à la formation de granules de stress et à la localisation des mRNPs dans le cytoplasme. Finalement, un modèle de diffusion facilité a été développé pour expliquer l'assemblage des microtubules orchestré par les polyamines naturelles (*telles que YB-1 qui sont positivement chargées dans la cellules*). L ensemble de ces données contribuent à une meilleure compréhension de processus biologiques fondamentaux concernant l assemblage de la tubuline en microtubules et le trafic des ARN dans la cellule. Ils pourraient avoir un intérêt pour développer de nouveaux médicaments qui ciblent les microtubules.