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# Atomic force microscopy reveals binding of mRNA to microtubules mediated by two major mRNP proteins YB-1 and PABP

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**Abstract A significant fraction of mRNAs is known to be associated in the form of mRNPs with microtubules for active transport. However, little is known about the interaction between mRNPs and microtubules and most of previous works were focused on molecular motor:microtubule interactions. Here, we have identified, via high resolution atomic force microscopy imaging, a significant binding of mRNA to microtubules mediated by two major mRNP proteins, YB-1 and PABP. This interaction with microtubules could be of critical importance for active mRNP traffic and for mRNP granule formation. A similar role may be fulfilled by other cationic mRNA partners.**


*Keywords:* Microtubule; RNA; RNP; Atomic force microscopy; YB-1; PABP

## 1. Introduction

In the cytoplasm, mRNAs interact with numerous protein partners [1–5], which enable the packaging of mRNAs into mRNPs and participate in the mRNA translational regulation, protection and anchoring to cytoskeleton [6]. Because of hindered diffusion in the cytoplasm [7], the efficient traffic of mRNPs is possible only through active transport on the cytoskeleton and particularly along microtubules by molecular motors [1]. In agreement with this, fluorescence tracking of mRNA shows probabilistic movements made of pauses, directional slidings and periods of free diffusion after detachments [8–10]. As detachments from microtubules slow down transport kinetics, transported mRNPs are thought to stay within a short distance from microtubules by an unclear mechanism [9]. An interesting explanation is that several motors interact with microtubules to ensure a longer lifetime of mRNP binding [9,11,12]. However, the implication of other mechanisms remains to be tackled.

Recently, a large scale identification of tubulin and microtubule binding proteins showed that 21% of them were known mRNA binding proteins like PABP [13]. Previous studies also demonstrated that mRNA binding partners were good candidates for tubulin binding [14]. Most of the mRNP proteins, especially the core mRNP proteins like YB-1, are highly cationic (p$I \sim 9.5$ for YB-1) [15]. Consequently, they are prone to interact with highly anionic microtubules (charge per tubulin at neutral pH being about 20–30$e^-$). In regard to this, we wondered whether cationic mRNP proteins can induce a relevant mRNP binding to microtubules. Here, we show by AFM imaging that two major core mRNP proteins (YB-1 and PABP) provoke an attraction between mRNPs and microtubules. The mechanism of this attraction was also further analyzed by gel shift and co-precipitation assays. We then suggest that mRNA binding partners other than molecular motors could facilitate the active transport of mRNPs and mRNA granule formation.

## 2. Materials and methods

Unless stated otherwise, all chemicals used in this work were purchased from Sigma–Aldrich (France).

### 2.1. YB-1 and PABP purification

Recombinant YB-1 and N-terminal His-tagged PABP were expressed in *Escherichia coli* and purified as previously described in [16,17], respectively. Purified proteins were dialyzed against 200 mM NaCl, 20 mM HEPES–KOH, pH 7.6, 1 mM DTT and stored at −80 °C.

### 2.2. Synthesis of mRNA

Plasmids pET-28a-α-globin and pSP72-2Luc were used as templates for synthesis by T7 polymerase of α-globin (660 nt) and 2Luc mRNAs (3000 nt), respectively [18]. After transcription, unincorporated NTPs were removed by gel-filtration through a NAP-5 column (GE Healthcare) and mRNAs were further isolated with RNAble (Eurobio) following manufacturer's recommendation.

### 2.3. Tubulin purification, microtubule preparation

Tubulin was purified from sheep brain using the method by Castoldi and Popov [19] and stored at −80 °C in 50 mM MES–KOH, pH 6.8, 0.5 mM dithiothreitol, 0.5 mM EGTA, 0.25 mM $MgCl_2$, 10% glycerol, and 0.1 mM GTP. Before use, tubulin stock was thawed and an additional cycle of polymerization was performed.

For microtubule assembly, 20 μM tubulin was incubated in 200 μl of assembly buffer (50 mM MES–KOH, pH 6.8, 1 mM EGTA, 5 mM $MgCl_2$, 20% glycerol, 1 mM GTP) containing 20 μM taxol for 15 min at 37 °C. Microtubules were then pelleted at $25000 \times g$ for 10 min at 37 °C and gently resuspended in a starting volume of assembly buffer.

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*2.4. RNP sample preparation*

YB-1:mRNA or PABP:mRNA complexes were first preformed in the assembly buffer for 10 min at 37 °C. Preformed microtubules were then added to the samples at various concentrations, and the mixtures were incubated for 10 min at 37 °C.

*2.5. Atomic force microscopy*

Atomic force microscopy is a powerful tool to study biomolecules, especially DNA [20] and RNA [21]. Its application to the imaging of microtubules is less common but some interesting studies were already published [22,23]. No special requirements are necessary to obtain the results presented in this work. Ten microliters of each sample was deposited on freshly cleaved mica and dried for AFM imaging as described by Pastré et al. [24]. The electrostatic adsorption of both protein:mRNA complexes on mica was then mediated by divalent magnesium cations. All AFM experiments were performed in intermittent mode with a multimode AFM instrument (Digital Instruments, Veeco, Santa Barbara, CA) operating with a Nanoscope IIIa controller. We used AC160TS silicon cantilevers (Olympus, Hamburg, Germany) with resonance frequencies of around 300 kHz. The applied force was minimized as much as possible. Images were collected at a scan frequency of 1.5 Hz and a resolution of $512 \times 512$ pixels.

*2.6. Native agarose gel electrophoresis*

Samples were loaded onto 0.6% agarose gels prepared using microtubule-stabilizing buffer (50 mM MES–KOH, pH 6.8, 1 mM EGTA, 2 mM $MgCl_2$, 10% glycerol) to avoid microtubule

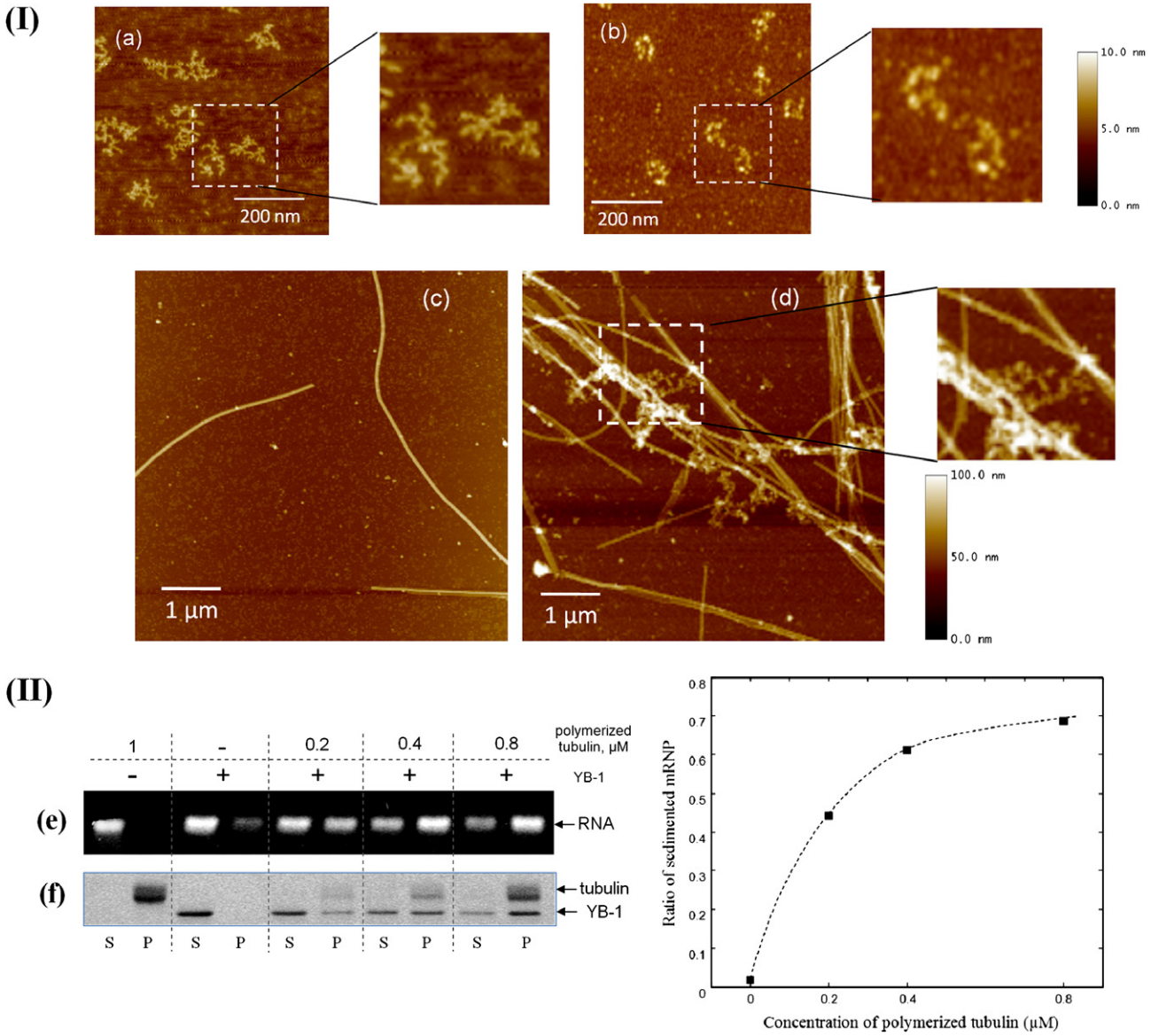


Fig. 1. (I) High resolution AFM imaging of RNP:microtubule association: (a) 2Luc mRNA (5 μg/ml). A proper spreading on mica revealed the secondary structure of RNA. (b) RNP particles obtained by mixing 5 μg/ml 2Luc RNA and 1.5 μM YB-1. The typical beads-on-string structure of the YB-1:RNA complexes was easily distinguished. (c) 2Luc RNA (5 μg/ml) in the presence of microtubules (0.8 μM polymerized tubulin) showing that microtubules and mRNA did not significantly interact with each other. (d) RNPs (5 μg/ml of RNA) in the presence of microtubules (0.8 μM polymerized tubulin). RNPs clearly co-localized with microtubules and tended to cluster on microtubule walls. In addition RNPs can also cross-link microtubules to form bundles. (II) Co-sedimentation assays with α-globin mRNA (5 μg/ml) and microtubules in the presence or absence of 1.5 μM YB-1 (R = 1/10). (e) mRNA staining with Sybr Green II. (f) Protein staining with Coomassie Blue. Microtubules increased the amount of RNPs in the pellet in a dose dependent manner, which clearly indicated that RNPs were bound to microtubules. The experiments were performed in assembly buffer (50 mM MES–KOH, pH 6.8, 1 mM EGTA, 5 mM $MgCl_2$, 20% glycerol, 1 mM GTP).