The X-chromosome-linked intellectual disability protein PQBP1 is a component of neuronal RNA granules and regulates the appearance of stress granules

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The polyglutamine-binding protein 1 (PQBP1) has been linked to several X-linked intellectual disability disorders and progressive neurodegenerative diseases. While it is currently known that PQBP1 localizes in nuclear speckles and is engaged in transcription and splicing, we have now identified a cytoplasmic pool of PQBP1. Analysis of PQBP1 complexes revealed six novel interacting proteins, namely the RNA-binding proteins KSRP, SFPQ/PSF, DDX1 and Caprin-1, and two subunits of the intracellular transport-related dynactin complex, p150Glued and p27. PQBP1 protein complex formation is dependent on the presence of RNA. Immunofluorescence studies revealed that in primary neurons, PQBP1 co-localizes with its interaction partners in specific cytoplasmic granules, which stained positive for RNA. Our results suggest that PQBP1 plays a role in cytoplasmic mRNA metabolism. This is further supported by the partial co-localization and interaction of PQBP1 with the fragile X mental retardation protein (FMRP), which is one of the best-studied proteins found in RNA granules. In further studies, we show that arsenite-induced oxidative stress caused relocalization of PQBP1 to stress granules (SGs), where PQBP1 co-localizes with the new binding partners as well as with FMRP. Additional results indicated that the cellular distribution of PQBP1 plays a role in SG assembly. Together these data demonstrate a role for PQBP1 in the modulation of SGs and suggest its involvement in the transport of neuronal RNA granules, which are of critical importance for the development and maintenance of neuronal networks, thus illuminating a route by which PQBP1 aberrations might influence cognitive function.

INTRODUCTION

The polyglutamine-binding protein 1 (PQBP1) was originally identified as a protein binding to polyglutamine (poly(Q)) stretches and it has been shown to interact with the poly(Q) containing proteins ataxin-1, huntingtin and the neuronal transcription factor Bm2 in a length-dependent manner (1,2). Shortly afterwards, we have established that mutations in PQBP1 cause X-chromosome-linked intellectual disability (XLID) disorders, such as Sutherland-Haan and Hamel syndromes (3). Since then, we and several other groups have reported PQBP1 mutations in affected probands with Porteous, Renpenning and Golabi-Ito-Hall syndromes, and in affected males with periventricular heterotopia (4–12). In addition to intellectual disability, affected probands with these syndromes share similar clinical features, including microcephaly, leanness, short stature, small testes and specific facial dysmorphism (MIM 300463). The pathogenetic mechanism is currently unknown, but we have recently shown that patients with a PQBP1 mutation express truncated protein (13).

The PQBP1 protein is a widely expressed protein and has several functional domains. One of these is known as the WW domain, which mediates specific interactions with proline-rich protein regions. This domain was found to interact with RNA polymerase II (1) and with the nucleocytoplasmic shuttling splicing factor SIPP1 (previously known as WBP1)
and NpwBP). The missense mutation in the PQBP1 WW domain present in the family with Golabi-Ito Hall syndrome compromised the complex with SIPP1, resulting in decreased pre-mRNA splicing of a reporter gene (14). The polar amino acid-rich domain, containing a stretch of arginine alternating with aspartic acid or glutamic acid (DR/ER), which is truncated by the most commonly found PQBP1 deletion and insertion frameshift mutations, is responsible for binding the poly(Q) stretches of PQBP1-interacting proteins (2).

As part of our investigation into the cellular functions of PQBP1 and to better understand the pathogenesis of XLID and polyglutamine-linked neurodegenerative disorders, we have searched for novel PQBP1 protein partners. We have found that PQBP1 interacts with cytoplasmic RNA-binding proteins, which have previously been shown to play a functional role in RNA processing, neuronal RNA transport and local translation. Furthermore, we have observed that PQBP1 co-localizes with these interaction partners in distinct cellular compartments.
RESULTS

PQBP1 interacts with proteins implicated in cytoplasmic RNA metabolism

To obtain a better understanding of cellular PQBP1 functions and the signalling pathways it is involved in, we searched for novel protein-binding partners. We applied a pull-down strategy for isolation of the PQBP1 complex using a neuronal cell line stably expressing c-myc-PQBP1. Co-immunoprecipitated proteins were stained with Coomassie blue (Fig. 1A) and analyzed by electrospray ionization time-of-flight (ESI-TOF) mass spectrometry. Eleven candidate interactors were identified, including the known PQBP1 interactor SIPP1 (NpwBP) (16). We selected the RNA-binding proteins K-homology (KH)-type splicing regulatory protein (KSRP), polyryrimidine tract-associated splicing factor (PSF), cell cycle-associated protein 1 (Caprin-1) and DEAD box polypeptide 1 (DDX1), which are associated with neuronal RNA transport granules to confirm them as PQBP1-complex partners by co-immunoprecipitation experiments.

KSRP (also known as FBP2) is a multi-functional RNA-binding protein that has been implicated in transcriptional regulation, neuro-specific alternative splicing and mRNA decay (17,18). It is the human homologue of rat MARTA1 and chicken ZBP2, which are both involved in neuronal transport and localization of β-actin mRNA in developing neurites (19,20). PSF is a multi-functional protein that participates in transcription regulation (21) and in neuro-specific splicing, and influences neuronal RNA transport (22). DDX1 is a neuronal adenosine triphosphatase (ATP)-dependent RNA helicase. It associates with poly(A) RNA (23), is present in RNA-transporting granules (22,24) and in SGs (25). Caprin-1 is a cytoplasmic phosphoprotein that is present in messenger RNPs in the brain (26), is involved in translation regulation together with fragile X mental retardation protein (FMRP) (27) and is recruited to SGs (28–30).

To confirm the association of PQBP1 with these proteins, we co-expressed PQBP1–enhanced yellow fluorescent protein (EYFP) together with V5-tagged Caprin-1, DDX1 or KSRP and PQBP1-hemagglutinin (HA) with V5-tagged PSF in COS7 cells. Interaction of proteins was analyzed by co-immunoprecipitation experiments using the respective antibodies. The immunoblot analyses verified all interactions (Fig. 1B–F). Following overexpression and immunoprecipitation of PQBP1 in frame with EYFP and other N-terminal or C-terminal tags several specific protein bands were obtained in western blot analyses (Figs 1B–F and 6 and data not shown), which migrate between 72 and 55 kDa in case of PQBP1 fused to EYFP. It is particularly the lower migrating PQBP1 protein band, which preferentially interacts with DDX1, KSRP and Caprin-1 (Fig. 1B–E). Because the PQBP1 interactors have been shown to bind mRNA, in parallel experiments we also investigated if the presence of RNA is critical for these protein–protein interactions. Co-immunoprecipitations were thus performed with cell extracts pre-incubated with RNase A (Fig. 1B–D). The immunoblot analyses showed that RNase treatment influenced the binding of Caprin-1, DDX1 and KSRP to PQBP1. A dissociation of PQBP1 from the protein complex was seen in RNase treated, but not in untreated cell extracts. For PSF-V5 treating, the cell extracts with RNase resulted in a dramatical change in the solubility of PSF-V5, which made comparison of co-immunoprecipitations with and without RNAse treatment impossible for this case. Still, we confirmed that overexpressed PQBP1 and PSF interact, as shown in Figure 1F, right panel. To corroborate this finding, we performed additional experiments using a specific antibody

![Figure 1](4918_Human_Molecular_Genetics_2011_Vol_20_No_24)

**Figure 1.** The PQBP1 complex contains DDX1, KSRP, Caprin-1 and PSF, and is dependent on the presence of RNA. (A) Cell lysates from a U373MG cell line stable expressing c-myc-PQBP1 were immunoprecipitated using anti-c-myc antibody or unspecific IgGs as control. Proteins were then loaded on an sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) gel and stained with Coomassie blue (upper panel). Bands were cut and analyzed by ESI-TOF mass spectrometry. 1/3 of the reactions was loaded on an SDS–PAGE gel, and after western blotting, the blot was probed with anti c-myc antibody to check the specificity of the immunoprecipitation (bottom panel). (B, C, D) PQBP1 interaction partners DDX1-V5 (B), KSRP-V5 (C) and Caprin-1-V5 (D) were overexpressed in COS7 cells either with PQBP1–EYFP or pEYFP-N1 vector. Cell lysates were treated with RNase A or RNase inhibitors followed by immunoprecipitation with anti-V5 antibody. Input lysates and immunoprecipitates were loaded on SDS–PAGE gels and western blots were probed with anti-V5 (upper panels, B–D) and anti-GFP antibodies (middle panels, B–D). Shorter exposures of blots probed with anti-GFP antibody are shown in the bottom panels (C, D). PQBP1–EYFP bands are significantly reduced in the RNase A-treated immunoprecipitations compared with the ones treated with RNase inhibitor. (E) Overexpression of PQBP1–EYFP in COS7 shows different protein bands ranging from 72 to 55 kDa. The lower migrating band (~55 kDa) preferentially co-precipitates with Caprin-1, DDX1 and KSRP (see also B–E). (F) PSF-V5 was overexpressed together with HA-PQBP1 in COS7 cells. Cell lysates were immunoprecipitated using anti-HA antibody or unspecific IgGs. Input lysates and immunoprecipitated proteins were loaded on an SDS–PAGE gel, the western blot was probed with anti-HA antibody (upper panel) and subsequently with anti-V5 antibody (bottom panel) to check the specificity of the immunoprecipitation (left panels). Cell lysate extracted from COS7 cells was immunoprecipitated using a specific antibody against PQBP1 (anti-N1-PQBP1) or unspecific IgGs (right panels). Input lysate and immunoprecipitated proteins were loaded on a SDS–PAGE gel, the western blot was probed with anti-PSF antibody (upper panel) and subsequently with anti-N1-PQBP1 antibody (bottom panel) to check the specificity of the immunoprecipitation. (G) Y2H analysis: yeast strain was transformed with expression constructs for PQBP1 as bait and PSF, DDX1, KSRP and Caprin-1 as prey. Isolated transformants were spotted on selective medium (SD4, left panel) or on a membrane for the beta-galactosidase assay (LacZ, right panel). Results for each prey are shown in quadruplicates. (H) PQBP1 interaction partner PSF-V5 was overexpressed in COS7 cells together with PQBP1–EYFP. Cell lysates were treated or not with arsenite followed by immunoprecipitation with anti-GFP antibody. Input lysates and immunoprecipitates were loaded on SDS–PAGE gels and western blots were probed with anti-V5-HRP (upper panel) and anti-GFP-HRP antibodies (bottom panel).
for immunoprecipitating endogenous PQBP1 and thereby established the interaction of PQBP1 with endogenous PSF (Fig. 1F, left panel). Additionally, we analyzed the interactions between PQBP1 and its partners Caprin-1, DDX1, KSRP and PSF by yeast two-hybrid (Y2H) experiments. These studies confirmed the results obtained by co-immunoprecipitation experiments, suggesting that PQBP1 directly interacts with these proteins in cells (Fig. 1G).

PQBP1 is seen in cytoplasmic granules, interacts with FMRP and associates with ribosomes

Immunofluorescence microscopy experiments revealed that endogenous as well as ectopically expressed PQBP1 is mainly present in the nucleus of mammalian cells (2,31). As PQBP1 is highly expressed in the central nervous system (CNS), especially in the cortex (32), we used primary cortical neurons from mouse embryos (E14.5) to study the distribution of PQBP1 in neuronal cells. Confocal microscopy analysis showed the presence of PQBP1 in the nucleus and the cytoplasm (Fig. 2A). Interestingly, cytosolic staining appeared as punctate structures in the perikaryon and in dendrites, as PQBP1 co-localized with the dendritic marker MAP2 (Fig. 2A). In addition to the dendritic localization, PQBP1 also showed a punctate structure in some axons, as there was a co-localization with the axonal marker TAU-1 (Fig. 2B). To analyze whether these PQBP1 dots could be synapses, we performed co-staining with the postsynaptic...
marker PSD95, but we did not detect clear overlapping signals of PQBP1 and PSD95 in cortical neurons (Fig. 2C).

As the newly found PQBP1 interaction partners have previously been shown to localize in neuronal RNA granules, we investigated the cellular distribution of Caprin-1, DDX1, KSRP and PSF in cortical neurons in the presence of PQBP1. For this, we transfected PQBP1–EYFP together with Caprin-1-cherry, or V5-tagged KSRP, PSF and DDX1 proteins (Fig. 3A–D). Co-expression of PQBP1–EYFP with all binding partners showed overlapping immunofluorescence signals in a punctated pattern in neuronal processes. Having found that the PQBP1 complex is dependent on the presence of RNA and that these complexes appear as microscopically visible dot-like structures in neurons, we explored the idea that these complexes might be RNA granules. Cellular RNA was labelled with ethidium bromide (EtBr) as previously described (33). As control, cortical neurons were treated with RNase prior to labelling. Specificity for RNA staining was indicated by the abolished EtBr signal following RNase treatment. In the absence of RNase, most PQBP1-positive granules overlapped with the RNA staining in neurons (Fig. 4A).

Given that intellectual disability is also a prominent clinical feature in patients with FMR1 mutations, we were interested in potential associations between PQBP1 and FMRP in neuronal granules. Using confocal microscopy, some cytoplasmic dots showed co-localization of PQBP1 and FMRP in neurons, suggesting that both proteins are present together in a subset of granules (Fig. 4B). Subsequent immunoprecipitation experiments performed using cell lysates from COS7 cells confirmed the interaction between endogenous PQBP1 and FMRP protein (Fig. 4C).

As PQBP1 interacts and co-localizes with FMRP in neuronal granules and RNA granules contain also ribosomes (34), we analyzed an association of PQBP1 with ribosomes, which has already been shown for FMRP (35). We purified ribosomes from F11 cells stably expressing PQBP1-V5 by ultracentrifugation on discontinuous sucrose gradients (Fig. 5). Part of the PQBP1-V5 protein was present in the ribosomal pellet (P), which also contained the ribosomal protein S3A. After treatment of the lysates with an ionic detergent (0.5% DOC) or high salt concentration (0.5 M KCl), which both dissociate ribosomes, PQBP1 as well as FMRP were removed from the ribosomal pellet (P), whereas the integral ribosomal protein S3A was still present. From these results, we conclude that a fraction of PQBP1 associates with ribosomes, but that PQBP1 is not an integral part of the ribosomes.

**PQBP1 associates with the dynactin complex**

In the search for novel-binding partners of PQBP1, we also carried out a Y2H screen using a human fetal brain cDNA library for the expression of prey proteins. Our screen revealed that the proteins p150Glued (also known as DCTN1) and p27 (DCTN6) interact with PQBP1 (data not shown). These proteins are subunits of the dynactin complex, which—together with the motor proteins dynein or kinesin—drives bidirectional microtubule-based transport of mRNPs and organelles (15). The main dynactin subunit, p150Glued, binds to microtubules as well as the motor protein. The smaller subunit p27 is part of the pointed-end complex and binds cargo (36).

As the motor proteins kinesin and dynein are involved in the neuronal transport of RNA granules (22,37,38), we examined the association of PQBP1 with p150Glued and p27 in

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**Figure 3.** PQBP1 and the newly found binding partners co-localize in cytoplasmic granular structures in primary neurons. Confocal images of cortical primary mouse neurons (E14.5) transfected with PQBP1 together with its newly identified partners. (A) PQBP1–EYFP (green) co-localizes with Caprin-1-cherry, (B) KSRP-V5 and (C) PSF-V5 (all shown in red) in cytoplasmic dots. (D) PQBP1-cherry (red) shows overlapping signals with DDX1-V5 (green) in granular structures. Some sections are enlarged. Heat maps show intensity of the signals. Z, Z-axis of confocal images. DIC, differential interference contrast.
mammalian cells. A 8-residue FLAG epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (FLAG)-tagged p27 fusion protein was co-expressed with either EYFP- or HA-tagged PQBP1, and protein complexes were co-immunoprecipitated from cell extracts. We found that PQBP1 indeed interacts with p27, confirming the results from the Y2H experiments (Fig. 6A). We also confirmed the interaction of endogenous p150Glued with FLAG-PQBP1 by co-immunoprecipitation experiments (Fig. 6B).

We next assessed whether p150Glued and p27 co-localize with PQBP1 in cortical neurons. Using confocal microscopy, we observed overlapping signals of PQBP1-cherry and FLAG-p27 in dendrites (Fig. 6C). A similar result was also obtained with the dynactin subunit p150Glued and PQBP1-cherry (Fig. 6D).

Using a cell-free microtubule co-sedimentation assay, we analyzed whether PQBP1 associates with microtubules. Protein lysates prepared from neuronal F11 cells stably expressing PQBP1-V5 were incubated with or without purified, stabilized microtubules in the presence of GTP and taxol. After a first ultracentrifugation, the microtubule pellet was analyzed by immunoblotting (Fig. 6E). In comparison to the control (without addition of microtubules), a larger amount of PQBP1-V5 was detected in samples with taxol-stabilized microtubules, indicating that PQBP1-V5 associates with microtubules. To test the specificity of this association, we resuspended the microtubule pellet after a first ultracentrifugation step and added either 0.5 mM ATP or 0.3 M KCl for 45 min to the samples (Fig. 6E). ATP induces movement of motor proteins, and high salt destroys the binding of motor proteins to microtubules. Both treatments resulted in the detachment of the motor proteins from microtubules. After an additional centrifugation step, we analyzed the resulting pellets (P2) and supernatants (S2) by immunoblotting, and PQBP1-V5 was detected in both fractions. In untreated fractions, PQBP1-V5 associated mainly with the microtubule pellet, whereas in ATP- and KCl-treated fractions, a bigger fraction of PQBP1 remained in the supernatant, indicating that binding of PQBP1 to microtubules is dependent on the association of motor proteins with such structures, as it is ATP- and high salt-sensitive.

Together, these results show a motor protein-dependent association of PQBP1 with taxol-stabilized microtubules, probably occurring via PQBP1 binding to the dynactin complex containing the main subunit p150Glued and the cargo-binding subunit p27. Moreover, our studies show that PQBP1 co-localizes with these proteins in punctate-like structures, which are detected in the processes of cortical neurons.

**PQBP1 is recruited into TIA-1-positive SGs in the cytoplasm and modulates their assembly**

SGs are cytoplasmic RNA–protein complexes that are induced by oxidative stress and share many components with neuronal RNA granules (39). SGs have been previously shown to contain the novel PQBP1 interaction partners Caprin-1, DDx1 and KSRP (25,29,30,40), suggesting that PQBP1 may also be present in these structures. We therefore investigated the cellular distribution of PQBP1 in response to oxidative stress.

**Figure 4.** PQBP1 co-localizes with RNA and FMRP in primary neurons. (A) Confocal images show overexpressed PQBP1-EYFP (green) with a dotted distribution in primary cortical neurons. Treatment with EtBr effectively labels the cellular RNA (red) as the EtBr signal is abolished by pretreatment with RNase. Confocal images were taken with the same settings for all exposures. Sections 1 and 2 are enlarged (bottom). PQBP1-EYFP dots (green) show also RNA staining (see crosslines in sections 1 and 2). Z stacks of the confocal images are shown on the right and on top of the enlarged sections. (B) Endogenous PQBP1 (green) and FMRP (red) appear in granular structures in the cytoplasm of primary neurons. A fraction of the cytoplasmic dots showed co-staining of PQBP1 and FMRP (arrows). Nuclei are stained in blue with DAPI. DIC, differential interference contrast. (C) PQBP1 interacts with FMRP. COS7 cell lysate was immunoprecipitated with a specific antibody against PQBP1 (anti-N1-PQBP1) or with unspecific IgGs. Input lysate and immunoprecipitated proteins were loaded on a SDS–PAGE gel, the western blot was probed with anti-FMRP antibody (upper panel) and subsequently with anti-N1-PQBP1 antibody (bottom panel) to check the specificity of the immunoprecipitation.
To induce the formation of SGs, neuronal F11 cells were treated with 0.5 mM arsenite for 50 min (41) and cells were subsequently immunostained for endogenous PQBP1 and TIA-1, a well-known marker of SGs. We found that PQBP1 was recruited into large TIA-1-positive aggregates that appeared in the perinuclear region of the cells, indicating that it associates with SGs (Fig. 7A). We observed that the arsenite-induced clusters contain both TIA-1 and endogenous PQBP1. Moreover, a similar result was obtained when stressed neuronal F11 cells stably expressing PQBP1-V5 were analyzed (data not shown). Next, we examined whether the PQBP1 interaction partners Caprin-1, DDX1 and KSRP are also present in PQBP1-containing SGs. As shown in Figure 7B, PQBP1 as well as the novel-binding partners described here are components of the same SGs, further supporting our hypothesis that these proteins are involved in similar cellular processes. To understand if the induced stress could compromise the PQBP1 protein complex, we

Figure 5. PQBP1 associates with ribosomes. Ribosomes from PQBP1-V5 stably expressing F11 cells were purified by ultracentrifugation on discontinuous sucrose gradients. The input (L, lysate), three fractions (F1–F3) corresponding to the top of the sucrose layer (F1), the 20% sucrose layer (F2), the 50% sucrose layer (F3) and the ribosomal pellet (P) were analyzed by SDS–PAGE followed by western blot probing for PQBP1-V5 (anti-V5), the ribosomal protein S3A (anti-S3A) and FMRP (anti-FMRP). Treatment of the lysates with 0.5% DOC or 0.5 M KCl resulted in dissociation of PQBP1 from the ribosomes.

Figure 6. PQBP1 interacts with components of the dynactin complex. (A) The small dynactin subunit FLAG-p27 tagged was overexpressed in COS7 cells together with HA-PQBP1 or PQBP1-EYFP. Cell lysates were immunoprecipitated with either anti-HA or anti-FLAG antibodies or unspecific IgGs. Input lysates and immunoprecipitated proteins were loaded on an SDS–PAGE gel and the western blots were probed with anti-HA or anti-GFP-HRP (upper panel, A and B) and anti-FLAG-HRP antibody (bottom panel). (B) FLAG-PQBP1 was overexpressed in COS7 cells. Protein cell lysate was immunoprecipitated with anti-FLAG or IgG agarose beads. Input lysates and immunoprecipitates were analyzed by western blotting and the blots were probed with anti-p150Glued. The experiments were performed in the presence of RNase inhibitors. (C and D) PQBP1 co-localizes with components of the dynactin complex. (C) PQBP1-cherry co-localizes with FLAG-p27 and with p150Glued (D) in cortical neurons (crosslines and arrows). Heat maps show intensity of the signals. (E) PQBP1 associates with microtubules. After ultracentrifugation of the pellets from neuronal F11 cell stably expressing PQBP1-V5, a co-sedimentation assay with taxol-stabilized microtubules was performed. PQBP1-V5 co-sediments with polymerized microtubules (upper row), as PQBP1-V5 is detected in the microtubule pellet (MT-pellet). In a second step, the MT-Pellet was resuspended, treated either with 0.5 mM ATP or with 0.3 M KCl for 45 min and centrifuged again (Pellet 2/ P2, lower row). Additions of ATP or high salt concentration lead to a detachment of the motor proteins from the microtubule (controls).
Figure 7. Endogenous PQBP1 co-localizes in SGs with newly identified binding partners and with FMRP. (A) Confocal images of neuronal F11 cells show PQBP1 (anti-C-PQBP1, green, second row) in SGs (arrows) induced by arsenite treatment (0.5 mM, 50–60 min) and visualized by the SG marker TIA-1 (red). Non-treated neuronal F11 cells served as a control (first row). (B) PQBP1 (anti-C-PQBP1, green) co-localizes with the binding partners Caprin-1-V5 (red, first row), DDX1-V5 (red, second row) and KSRP-V5 (red, third row) in SGs (arrows) induced by arsenite treatment in neuronal F11 cells. Nuclei are stained in blue with DAPI. (C) Immunofluorescence experiments show partial co-localization (arrows) of PQBP1 (anti-C-PQBP1, green) with FMRP (red) in SGs, after their induction with arsenite (0.5 mM, 50–60 min) in neuronal F11 cells (first row) and in primary cortical neurons (second row). Nuclei are stained in blue with DAPI.
performed immunoprecipitation experiments after arsenite treatment. The results indicate that PQBP1 and PSF still interact using these conditions (Fig. 1H).

We next assessed whether PQBP1-associated SGs are also positive for the protein FMRP, which was previously found in SGs (42). We found that arsenite treatment of neuronal F11 cells and primary cortical neurons induced formation of FMRP-containing SGs that also contain endogenous PQBP1 (Fig. 7C, see arrows).

It is well known that some SG proteins, such as TIA1, G3BP or Caprin-1, promote the assembly of SGs when they are overexpressed. To evaluate the importance of PQBP1 in SG formation, we overexpressed PQBP1 in COS7 cells and after arsenite treatment, we analyzed and quantified SG appearance in untransfected and transfected cells. After induction of oxidative stress, ~96% of untransfected cells were SG positive, containing two or more SGs with a diameter of at least 1 μm (Fig. 8). A similar result was obtained for cells in which overexpressed PQBP1 was present exclusively in the nucleus (data not shown). In contrast, in cells exhibiting a nuclear and cytoplasmic localization of overexpressed PQBP1, the number of SG-positive cells was significantly reduced (to around 26%). We further investigated the effect of disease-associated mutated forms of PQBP1 on the appearance of SGs under stress conditions. Three disease-associated mutants carried frameshift deletions or insertions in the DR/ER repeat, which is essential for the interaction with the poly-glutamine tract (2). Following overexpression, these mutant proteins localized to both the nucleus and the cytoplasm (3). Arsenite treatment of cells overexpressing these mutants resulted in a reduced appearance of SG-positive cells between 62% and 69% compared with untransfected cells. However, this effect was less pronounced than in cells with wild-type cytoplasmic protein (26%). In addition, we tested two PQBP1 mutations which did not truncate the DR/ER repeat. Both mutants showed a comparable effect on SG formation as the wild-type PQBP1 (27% and 31%). Thus, our results indicate that cytoplasmic PQBP1 with an intact poly(Q) binding DR/ER repeat significantly influences the appearance of SGs in mammalian cells, while mutant forms with a truncated poly(Q) binding region are less efficient.

DISCUSSION

Mutations in the gene encoding the PQBP1 cause X-linked intellectual disability (3–12). In addition, this protein has been linked to different progressive neurodegenerative diseases (1,2,43), suggesting that it influences poly(Q)-mediated protein misfolding and toxicity. To date, little is known about the cellular functions of PQBP1, except for its potential involvement in nuclear RNA processes, such as transcription and splicing (1,14).

Our data suggest, for the first time, that PQBP1 plays a functional role in cytoplasmic RNA metabolism in neurons. This is supported by the following observations: first, PQBP1 binds RNA-binding proteins with an established role in cytoplasmic RNA localization and translation regulation. Secondly, PQBP1 is present in cytoplasmic granular structures in primary cortical neurons, which stain positive for RNA. Thirdly, PQBP1 binds two key proteins of the motor protein-related dynactin complex. Fourth, PQBP1 is present in SGs induced by oxidative stress and influences the appearance of SGs in mammalian cells.

PQBP1 interacts with RNA-binding proteins that co-localize with cytoplasmic RNA granules

PQBP1 is present in the nucleus and in punctate, granular structures in the cytoplasm, especially in primary neurons. In addition, in cortical neurons, we detected the protein in dendrites and also some axons, suggesting a novel role for PQBP1 in the neuronal cytoplasm. Pulldown experiments enabled us to identify novel interactions between PQBP1 and several RNA-binding proteins, including KSRP, PSF, DDX1 and Caprin-1. Similar to PQBP1, these interactors have established nuclear functions, e.g. PSF is a splicing factor (44), and KSRP is involved in transcription regulation, alternative splicing and mRNA decay (18,45,46). In addition, KSRP, PSF, DDX1 and Caprin-1 have been identified in cytoplasmic RNA-transporting granules and are involved in cytoplasmic localization and/or translational control of certain mRNAs in neurons (22,26,47). These findings raised the intriguing possibility that PQBP1 is a component of neuronal RNA granules and could thus play a role in neuronal cytoplasmic RNA metabolism. Co-immunoprecipitation studies demonstrated that the binding of PQBP1 to KSRP, DDX1 and Caprin-1 was dramatically decreased in the absence of RNA, suggesting that RNA is critical for protein complex formation. Caprin-1 contains RGG motifs which are characteristic of RNA-binding domains and these could play a role in RNA binding of the PQBP1 complex. Together, these results...
indicate the presence of a PQBP1 complex that contains RNA-binding proteins and RNA, suggesting that disease-associated mutations in PQBP1 could disrupt such complexes in patients with XLID.

PQBP1 is a member of RNA granules and interacts with proteins of the dynactin complex

Since the PQBP1 interaction partners described here have been previously shown to be part of certain types of RNA granules, we analyzed their combined subcellular localization in cortical neurons. Interestingly, in some studies, these interaction partners have been found in the same types of granules, e.g. KSRP, Caprin-1 and DDX1 were present in β-actin-enriched RNA granules (47), whereas PSF and DDX1 were identified together with mRNAs for CaMKIIα and ARC in a large RNase-sensitive RNA-transporting granule (22). All four novel-binding partners exhibited a punctate co-localization with PQBP1 in neurites, indicating that PQBP1 is a component of granules that also harbour Caprin-1, DDX1, KSRP and PSF. In addition, the neuronal PQBP1-containing granules co-localized with RNA, suggesting that these structures could be RNA granules. Thus, PQBP1 and its interaction partners might regulate neuronal cytoplasmic RNA metabolism, including the regulation of cytoplasmic mRNA transport, mRNA localization and local translation. This idea is supported by the finding that PQBP1 interacts with the proteins p150Glued and p27 of the dynactin complex. As mentioned previously, dynactin serves as an adaptor between the transported cargo and several motor proteins, and it is involved in bidirectional mRNA transport (15,48). p150Glued binds directly to microtubules and to motor proteins, whereas p27 is part of the cargo-binding domain of the dynactin complex (36,49). p150Glued and p27 interact with PQBP1 and both proteins co-localize with PQBP1 in cortical neurons, particularly at the tips of neurites. These results suggest that PQBP1 could serve as a molecular adaptor protein that associates with trafficking particles, such as RNA granules, and with the dynactin-motor protein complex and in this way could regulate RNA transport in neurons. Further support for the role of PQBP1 as an adaptor molecule comes from the motor protein-dependent microtubule association of PQBP1, which presumably occurs via the dynactin interaction.

Interestingly, an important function in neuronal RNA transport has also been attributed to the fragile X mental retardation syndrome-associated protein FMRP (50). FMRP is an RNA-binding protein involved in several steps of RNA metabolism such as RNA transport and translation regulation. In neurons, it plays a role in dendritic transport of specific RNAs and regulates compartmentalized protein synthesis in synapses in response to stimuli (51). Loss of FMRP leads to abnormal spine development and cognitive dysfunction, presumably via a misregulated transport and/or local translation at synapses (51,52).

Therefore, it was obvious to investigate whether PQBP1 and FRMP co-localize in neurons. We detected a partial co-localization of these proteins in cytoplasmic granules and could show an interaction of the two endogenous proteins, which indicates the potential of overlapping functions for these proteins in specific cellular compartments during neuronal development and in cognition and places PQBP1 among other RNA-binding proteins implicated in intellectual disability, including AF4/FMR2 (53), VCX-A (54), UPF3B (55) and ZC3H14/Nab2 (56). A model showing the novel PQBP1 interactors and suggested functions of the cytoplasmic PQBP1 complex is shown in Figure 9. Additional evidence that cytoplasmic RNA–protein complexes and neuronal transport play an important role in synaptic plasticity, and neuronal development was provided by a recent report on how the c-Jun N-terminal kinase (JNK) pathway regulates RNA transport and also the cellular stress response (57). Truncation of the protein JNK3, which is exclusively expressed in the CNS, leads to a seizure-associated mental disorder (58). Given the link between several disease genes and RNA transport, deciphering the transport mechanism may help us to unravel the pathophysiology of intellectual disability. Our proposed link between PQBP1 and p150Glued may also help explain why transgenic mice overexpressing human PQBP1, mice with a disrupted dynactin complex and mice heterozygous for a DCTN1 (p150Glued) missense mutation all show a late-onset and gradually progressive motor neuron disease phenotype and neuropathologies similar to the devastating neurodegenerative disorder amyotrophic lateral sclerosis (ALS; MIM 105400) (59–62). Interestingly, DCTN1 mutations were also found in patients suffering from an ALS-related motor neuron disease (63,64), or a slowly progressive distal hereditary motor neuronopathy with vocal paresis (65). The role of PQBP1 as well as the functional significance of the interactions for the pathophysiology of patients with XLID remains to be established in the future.

Figure 9. Model for a possible cytoplasmic function of PQBP1. PQBP1 interacts with DDX1 and probably via its polyQ-binding region DR/ER with the polyQ-rich proteins Caprin-1, PSF, KSRP and FMRP, dependent on the presence of RNA. Furthermore, PQBP1 co-localizes with its binding partners in cytoplasmic neuronal granules, as well as with RNA. PQBP1 binds p27 and p150Glued of the dynactin complex and associates with microtubules via the motor proteins. PQBP1 might therefore serve as an adapter between the dynactin-motor protein complex and RNA granules.
PQBP1 assembles into SGs and regulates their formation

We have also shown here that PQBP1 is recruited into TIA-1-positive SGs, which are induced by oxidative stress. SGs form in the cytoplasm during conditions in which translation is aborted or reduced. They are thought to protect the cell from stressful conditions by transiently recruiting mRNAs from stressful conditions by transiently recruiting mRNAs and associated proteins into these structures, to be stored or degraded (66). Oxidative stress can influence a number of important cellular pathways, including synaptic plasticity (67). It is interesting to note that many neurological diseases are linked to oxidative stress, including neurodegenerative diseases (68) as well as intellectual disability in Down syndrome (69), Rett syndrome (70), autism (71) and Fragile-X syndrome (72). How PQBP1 is recruited to SGs needs further investigation. The ID protein WDR62, which is a JNK-scaffolding protein is recruited to SGs together with activated JNK (73,74). The XLID protein RSK2 is recruited into SGs via its direct interaction with the SG marker TIA-1 (75). Several of the SG-nucleating proteins have glutamine-rich domains, such as TIA-1, TIAR, G3BP, CPEB (41) and also Caprin-1 and KSRP. PQBP1, which is a poly(Q)-binding protein, might associate with glutamine-rich proteins and thereby could be integrated into SGs.

Overexpression of many SG components such as FMRP and Caprin-1 induce formation of SGs (30,41). Remarkably, this does not seem to be the case for PQBP1. Interestingly, in stressed cells overexpressed nuclear PQBP1 had no effect on the appearance of SG-positive cells, whereas ectopic cytoplasmic PQBP1 resulted in a significantly lower number of cells with SGs. Of note, this reduction was much weaker in cells overexpressing disease-associated PQBP1 mutants with a truncated polyglutamine-binding DR/ER repeat region. In contrast, PQBP1 mutants with an intact DR/ER repeat showed the same reduction as wild-type PQBP1. These results suggest that cytoplasmic PQBP1 is a potential negative regulator of SGs and that its poly(Q) binding DR/ER repeat region plays a role in SG formation or disassembly. On the same path, Staufen-1 is involved in recovery from stress by stabilizing polyribosomes and protecting them from stress-induced breakdown (76). MK-STYX has been suggested to inhibit SG formation through interacting with G3BP, which, dependent on its endoribonuclease activity, induces self aggregation and promotes SG assembly after stress stimuli. A mutant of MK-STYX with impaired G3BP binding lost its ability to inhibit G3BP-induced SG assembly (77). On the contrary, FMRP plays a positive role in SG formation as the absence of FMRP or a disease-associated nonsense mutation resulted in reduced SGs (78).

PQBP1 is a nucleocytoplasmic shuttling protein that piggybacks with SIPP1 (79). Its subcellular distribution is dependent on SIPP1 and additional yet unknown factors. PQBP1 could play a role in the assembly or dissolution of SGs and could connect important cell responses, such as cell defense mechanisms, inflammation and cell survival. How precisely PQBP1 might contribute to SG dynamics remains to be determined. Many SG-nucleating proteins are known to have a poly(Q) repeat in their protein sequence (41). PQBP1 might regulate the SG appearance via poly(Q) binding with these SG-nucleating proteins. This might prevent these proteins from inducing SG assembly. In this context, we propose that disease-associated forms of PQBP1, in which the polyglutamine-binding domain are truncated, are less efficient than wild-type PQBP1 in binding poly(Q) proteins. Another possibility is that the modulatory effect of PQBP1 on SGs is mediated through its interaction with the dynactin complex. It has been shown that the activity of motor proteins is required for SG aggregation (41,80). In addition, recent studies revealed that motor proteins are critical for SG dynamics and that destruction of the dynactin complex results in a lower number of SGs in cells (81,82). Further studies with neuronal model systems will contribute to our understanding of how oxidative stress and other stress responses in the brain contribute to the pathophysiology of patients with a defective PQBP1 protein.

In summary, we have identified cytoplasmic PQBP1 protein complexes that are present in neuronal RNA granules and might act as adaptor molecules linking granules with the motor proteins. Upon stress, PQBP1 moves into SGs, together with FMRP and with its novel-binding partners Caprin-1, DDX1 and KSRP. Our findings provide the first evidence that PQBP1 may be a multifunctional protein with a role in regulating cytoplasmic signalling events involved in mRNA metabolism and thereby provide new insights into the pathogenesis of intellectual disability.

MATERIALS AND METHODS

Plasmid constructs

PQBP1 cDNA (from nucleotide pos. 255–1105, accession no. NM_005710) was amplified from control lymphoblastoid cell line RNA using primers PQBP1_for tacatGAATTCCgatcgcgtgcccgttgcg and PQBP1_rev tacatGTCGACgcaggatatcaccagaaagc, carrying EcoRI and SalI restriction sites, digested and cloned into pCMVTag3A vector in frame with a myc tag (Stratagene). The insert has been subsequently digested with the mentioned enzymes, and has been inserted in pCMVtag2A (Stratagene) to clone PQBP1. For cloning PQBP1 wild-type and c.194-A-G missense mutation in the pEYFP-N1 vector (Clontech), the complete open reading frame (ORF) was amplified from control and patient cDNA using primers PQBP1_Eco_for cagGTCGACgtgcgcggtcccctgcgcgtgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcgccggtcctgcg
10% FBS, 1 U373MG (Eagle’s minimal essential medium containing serum (FBS), F11 (Hams F12 containing 10% FBS) and becko’s modified Eagle medium containing 10% fetal bovine treated with L-glutamine and penicillin/streptomycin: COS7 [Dul-

For cell culture and treatment before (83).

Cell culture and treatment

Cells were maintained in the respective medium, supple-
mented with L-glutamine and penicillin/streptomycin: COS7 [Dul-
beco’s modified Eagle medium containing 10% fetal bovine serum (FBS)], F11 (Hams F12 containing 10% FBS) and U373MG (Eagle’s minimal essential medium containing 10% FBS, 1× non-essential AA, 1 mM sodium pyruvate).

All transient transfection experiments were performed with Lipofectamine 2000 (Invitrogen) according to the manufactur-
er’s recommendations. 3 × 10⁴ cells were seeded in 12 well plates and transfected with 0.8 μg plasmid DNA per well and 1 μl Lipofectamine, whereas 1 × 10⁶ cells were seeded in 150 cm² flask, transfected with 8 μg DNA per flask and 25 μl transfection reagent. After 24 or 48 h, cells were fixed for immunofluorescence microscopy analysis or harvested. To induce oxidative stress, cells were treated with 0.5 mM arsenite for 50–60 min at 37°C and then fixed as described in immunocytochemistry methods (see below).

Establishment of a U373MG cell line stably expressing c-myc-PQBP1

U373MG cells were seeded in six-well plates at a cell density of 1.5 × 10⁵ per well and incubated at 37°C for 24 h. Subcon-
fluent cells were transfected with 3 μg DNA per well either with c-myc-PQBP1 or pCMVTag3A vector, using 2 μl per well of Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, 100 μg/ml of G418 was added to the medium. Surviving individual colonies were picked and ampli-
fied in the selective medium.

F11 cells stably expressing PQBP1-V5

For establishing the F11 cell line stably expressing PQBP1-V5 (pLenti6-PQBP1-V5), we used the ViraPower™ Lentiviral Expression System according to the manufacturer’s protocol (Invitrogen).

Primary cortical mouse neurons

Primary mouse cortical neurons were isolated from brains of NMRI mouse embryos (embryonic day 14.5, E14.5). In brief, isolated and pooled embryonic cortices were dissociated by incubation with trypsin/ethylenediaminetetraacetic acid and trituration with glass pipettes in Neurobasal media (Gibco) supplemented with B27 (Gibco) and 0.5 mM L-glutamine (Lonza). 1.2 × 10⁵ cells were seeded onto glass slides coated with 0.2 mg/ml poly-D-lysine (Sigma) and 2 μg/ml laminin (Sigma) in 12-well plates. Cells were then incubated in a humidified incubator at 37°C and 8% CO₂. Primary neurons were transfected with 1.5 μg DNA and 2 μl Lipofect-
tamine 2000 (Invitrogen) per well at day 7 in vitro (DIV) and fixed at DIV 10 in parafomaldehyde (PFA) as described above for immunofluorescence.

Immunoprecipitation and western blot analysis

Cells were resuspended in 50 mM Tris pH 7.4, 100 mM NaCl, 5 mM MgCl₂ supplemented with Mini Complete Protease Inhibitor (Roche), and 0.1 mg/ml RNase A or 100 Units/ml of RNase inhibitors when indicated, and lyzed using 10 strokes of a 29 G needle. RNase treatment was performed for 15 min at 37°C. To induce oxidative stress, COS7 cells were treated with 0.5 mM arsenite for 50 min at 37°C and then lyzed as described above.

After preclearing the lysate with 25 μl protein G-agarose (Roche), we incubated the supernatants for 3 h or overnight with 2 μg of the specific antibody or 2 μg of mouse IgG (mlgG) or rabbit IgG (rlgG) as a negative control, by rocking at 4°C. Samples were then incubated with protein G-agarose by rocking for 1 h at 4°C. Alternatively, precleared lysates were incubated with 20 μl of anti-FLAG M2-agarose

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or anti-mouse IgG-agarose beads for 1 h. Afterwards, beads were washed three times with lysis buffer, followed by resuspension in 1× sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) buffer (3% SDS, 100 mM Tris–HCl pH 6.8, 10% glycerol, 0.2 mM DTT and bromophenol blue), boiled for 5 min and centrifuged at 10 000g for 1 min. Supernatants were separated by SDS–PAGE, followed by western blot analysis. Coomassie staining was performed using the Imperial Protein Stain solution (Pierce).

**Immunocytochemistry**

Cells were fixed for 10 min with 4% PFA in phosphate buffered saline (PBS), permeabilized in 0.2% Triton X in PBS for 5 min and incubated with 4% bovine serum albumin in PBS for 1 h at room temperature. They were then incubated overnight with the primary antibodies in the same solution at 4°C, washed three times with PBS and subsequently incubated with secondary antibodies in blocking solution, and again washed three times with PBS, dipped in water and mounted with Fluoromount and 0.5 μg (4',6-diamidino-2-phenylindole) DAPI/ml. Images were acquired using a Axioskop 2 (Zeiss) or a confocal laser-scanning microscope (Axiovert 200M, Zeiss LSM 510) with a ×63 objective and were analyzed with the ZEISS LSM Image Browser. To calculate the percentage of SG-positive cells, 100 untransfected cells and 100 transfected cells with a cytoplasmic localization of each PQBP1 construct were counted and analyzed in each of the three independent experiments. Cells with at least two SGs with a diameter of 1 μm were evaluated as SG positive. The results are presented as means ± SD.

**Discontinuous sucrose gradient**

F11 cells stably expressing PQBP1-V5 were lysed in TKM buffer (20 mM Tris, pH 7.4, 100 mM KCl, 5 mM MgCl2 in DEPC-H2O) and the lysates were cleared by centrifugation with 10 000g for 10 min in a table centrifuge. 2.5 mg of protein in 900 μl TKM buffer was analyzed in a discontinuous sucrose gradient containing a 20% sucrose layer (600 μl) on top of a 50% sucrose layer (500 μl) by ultracentrifugation (Beckman TL100 centrifuge with a Beckman TLA120.2 rotor) for 16 h at 4°C with 70 000g. Lysates were treated with 0.5% DOC or 0.5 mM KCl for 30 min at 4°C before ultracentrifugation. Same amounts of proteins of the layer without sucrose (Fraction 1), the 20% sucrose layer (Fraction 2), the 50% sucrose layer (Fraction 3) and the pellet (P) were analyzed by SDS–PAGE followed by western blot.

**Ethidiumbromide staining**

For RNA staining, primary neurons were fixed with 4% PFA in PBS for 20 min, washed with 0.1 mM Tris pH 7.4 for 5 min and permeabilized in 70% ethanol/DEPC water at 4°C overnight. Cells were incubated with 100 μg/ml of ethidium bromide in PBS for 1 h and then washed in 4xSSC/0.1% Tween20 at room temperature. As control, RNase treatment was performed with 1 μg/μl RNase A for 30 min at 37°C, before ethidiumbromide staining.

**Co-sedimentation assay with taxol-stabilized microtubules**

The co-sedimentation assay was performed as described in Goswami et al. (84). Taxol-stabilized microtubules have been formed from α-tubulin dimers (a kind gift from Dr Goswami) purified from porcine brain according to (85). In brief, F11 cells stably expressing PQBP1-V5 were lysed in modified PEM buffer (20 mM PIPES, pH 6.8, 1 mM EGTA and 0.2 mM MgCl2) and centrifuged for 30 min at 57 000g. The supernatant was incubated with or without taxol-stabilized microtubules for 45 min at 37°C followed by centrifugal separation of the microtubules (P1/MT-pellet, with MT-associated proteins) and free tubulin dimers (S1 supernatant, with non-associated proteins). The MT-pellet (P1, taxol-stabilized) was then resuspended in PEM buffer and incubated with either 0.3 mM potassium chloride or 0.5 mM ATP or without any additives for 45 min at 37°C. After centrifugation at 57 000g for 30 min, the resulting pellet (P2) was resuspended in the same volume as the supernatant (S2). Same volumes have been analyzed for the presence of PQBP1-V5 by western blotting.

**Antibodies**

Following antibodies were used: anti-C-PQBP1 ((13), polyclonal, rabbit, IF: 1:50), anti-N1-PQBP1 ((13), polyclonal, rabbit, WB:1:1000, IP: 10 μl), anti-V5 (Invitrogen, monoclonal, mouse, IF: 1:1000, WB: 1:5000, IP: 2 μg), anti-HA (Covance, monoclonal, mouse, WB: 1:5000), anti-GFP (Roche, monoclonal, mouse, IP: 2 μg), anti-GFP (Abcam, polyclonal, goat, WB: 1:5000), anti-GFP-HP (Abcam, polyclonal, goat, WB: 1:5000), anti-V5-HP (Invitrogen, monoclonal, mouse, WB: 1:3000), anti-FLAG M2 affinity gel (Sigma, monoclonal, mouse), anti-FLAG M2-HP (Sigma, monoclonal, WB: 1:10 000), anti-p150Glued (BD Transduction, monoclonal, mouse, WB: 1:2000) anti-MAP2 (Chemicon, monoclonal, rabbit, IF: 1:500), anti-TAU1 (Chemicon, monoclonal, mouse, IF: 1:500), anti-PSD95 (Stressgen, monoclonal, mouse, IF: 1:500), anti-TIA-1 (Sanata Cruz, monoclonal, goat, IF: 1:100), anti-FMRP (Chemicon, monoclonal, mouse, IF: 1:200, WB: 1:1500), anti-PSF (Sigma-Aldrich, monoclonal, mouse, WB: 1:1500), anti-Ataxin-2 (BD Transduction Laboratories, monoclonal, mouse, IF: 1:200), anti-S3A (gift from J. Stahl, polyclonal, goat (86), WB: 1:500). All HRP-conjugated antibodies (Santa Cruz) were used with a dilution of 1:5000 in 5% milk/PBST. Fluorescent labelled antibodies (anti-mouse-AlexaFluor488, anti-rabbit-AlexaFluor488, anti-mouse-AlexaFluor594, anti-rabbit-AlexaFluor594, anti-goat-AlexaFluor 594, anti-mouse-TexasRed) were purchased from Invitrogen and used with a dilution of 1:500.

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