Human Mpp11 J Protein: Ribosome-Tethered Molecular Chaperones Are Ubiquitous

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The existence of specialized molecular chaperones that interact directly with ribosomes is well established in microorganisms. Such proteins bind polypeptides exiting the ribosomal tunnel and provide a physical link between translation and protein folding. We report that ribosome-associated molecular chaperones have been maintained throughout eukaryotic evolution, as illustrated by Mpp11, the human ortholog of the yeast ribosome-associated J protein Zuo. When expressed in yeast, Mpp11 partially substituted for Zuo by partnering with the multipurpose Hsp70 Ssa, the homolog of mammalian Hsc70. We propose that in metazoans, ribosome-associated Mpp11 recruits the multifunctional soluble Hsc70 to nascent polypeptide chains as they exit the ribosome.

In the crowded cellular environment, folding of newly synthesized polypeptides into their active three-dimensional conformations requires the action of a class of proteins called molecular chaperones. These chaperones bind unfolded polypeptides by interacting with hydrophobic regions that are normally sequestered in the interior of a protein, thereby preventing aggregation and promoting folding (1, 2). Because partially synthesized nascent chains are particularly prone to aggregation, the presence of specialized molecular chaperones that interact directly with the ribosome near the polypeptide exit tunnel provides an important direct link between translation and protein folding (3). Such ribosome-tethered chaperones, only identified in microorganisms, are structurally divergent. Trigger factor, a member of the peptidyl-prolyl isomerase family, is found ubiquitously in bacteria (1). In contrast, the yeast ribosome-associated chaperone machinery is of the Hsp70:J protein type: Both the J protein Zuo and its Hsp70 partner Ssb independently associate with ribosomes (4, 5). In such systems, both a J protein and an Hsp70 are obligatory; the J protein serves to recruit the Hsp70 to substrate polypeptides as well as to stimulate Hsp70's adenosine triphosphatase (ATPase) activity, thus stabilizing Hsp70's interaction with the substrate (6, 7).

Identification of ribosome-associated chaperones in unicellular but not multicellular organisms raises the question of whether early involvement by molecular chaperones at the ribosome to prevent aggregation of nascent polypeptides is universally important (8, 9). As a candidate for a metazoan ribosome-associated chaperone, we chose the human protein, Mpp11, which is orthologous to the yeast ribosome-associated J protein Zuo and is present in a wide variety of eukaryotes (10, 11). In addition to having 45% identity in their J domains—the region of J proteins critical for functional interaction with Hsp70—Zuo and Mpp11 are 42% identical in a 80–amino acid region required for ribosome-association of Zuo (4).

To determine whether Mpp11 is ribosome associated, we separated lysates of human tissue culture cells by sucrose density gradient centrifugation and probed for Mpp11 (12). Mpp11 cosedimented with human ribosomal protein L10 (Rpl10) (Fig. 1A). Because Mpp11 is so highly conserved, we used rabbit reticulocyte lysate (RRL) to further assess ribosome association. After centrifugation of RRL through a sucrose cushion, the Mpp11 and Rpl10 cross-reactive proteins were quantitatively found in the pellet fraction (Fig. 1B). Because RRL has a high concentration of nontranslating ribosomes, this cofractionation suggested that Mpp11 interacts directly with the ribosome and does not require a nascent polypeptide chain for association. To test this idea more directly, ribosomes purified from RRL and stripped of endogenous Mpp11 by treatment with high salt were incubated with purified Mpp11 (Fig. 1C). When added at a stoichiometry of approximately 1:1, most Mpp11 sedimented with ribosomes; at a ratio of 2:1, Mpp11 distributed equally between the supernatant and pellet, consistent with the idea that Mpp11 bound directly to mammalian ribosomes.

Because both Mpp11 and Zuo associate with ribosomes, we asked if Mpp11 could substitute for Zuo when expressed in yeast. Mpp11 partially rescued the cation hypersensitivity of a Δzuo strain (13), as demonstrated by enhanced growth in the presence of NaCl or paromomycin (Fig. 2A and fig. S1). To assess whether Mpp11 was ribosome associated in the heterologous yeast system, lysates prepared from the Δzuo strain expressing Mpp11 were subjected to centrifugation through a sucrose cushion. Ribosomes were present in the pellet fraction, as was the vast majority of Mpp11 (Fig. 2B). The resuspended pellet was subjected to centrifugation through a sucrose density gradient. The distribution of Mpp11 in the gradient closely coincided with that of ribosomes, even after treatment with ribonuclease A (RNase A) caused ribosomes to migrate as 80S monosomes. Thus, nearly all of Mpp11 was associated with yeast ribosomes.

Because of the sequence similarity between Mpp11 and the region of Zuo required for ribosome association, we asked whether the two proteins interacted similarly with ribosomes. Ribosomes from wild-type cells and Δzuo cells expressing Mpp11 were incubated with various concentrations of KCl. The endogenous Zuo and heterologous Mpp11 showed similar salt sensitivities, with most of the protein
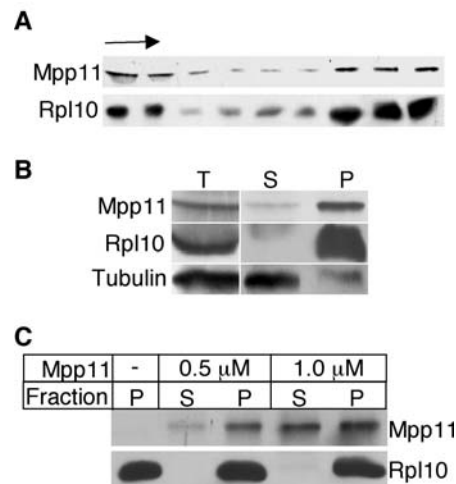

Fig. 1. Ribosome association of mammalian Mpp11. (A) Cell lysate from human embryonic kidney cells (HEK293) centrifuged through a sucrose gradient of 5 to 47%. Arrow denotes direction of the gradient. Fractions were collected and subjected to immunoblot analysis with antibodies directed against human Mpp11 or human Rpl10. (B) Ribosomes from RRL were isolated by centrifugation through a sucrose cushion. Equivalent amounts of supernatant (S) and ribosomal-containing pellet (P) from centrifugation of 5 μl of RRL (T) were subjected to immunoblot analysis with the indicated antibodies. (C) Indicated concentrations of purified Mpp11 were incubated in the presence of high salt–washed ribosomes (~0.5 μM). Samples were centrifuged and the supernatant (S) and pellet (P) fractions were analyzed as described above with the use of the indicated antibodies.

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being released from ribosomes after treatment with 200 mM KCl (Fig. 2C). To test the idea that the two proteins bind to the same site on the ribosome, we performed a competition assay. Ribosomes stripped of endogenous Zuo by treatment with high salt were incubated with approximately equimolar amounts of Zuo and increasing concentrations of Mpp11 (Fig. 2D). In the absence of Mpp11, nearly all of the Zuo was found in the pellet after centrifugation, indicative of its ribosome association. When Mpp11 was present in threefold excess, less than 20% of the added Zuo was ribosome associated, indicating that binding of Mpp11 and Zuo was mutually exclusive, consistent with the idea that Mpp11 and Zuo occupy the same, or overlapping, sites on the yeast ribosome.

Interaction of J proteins with the ATPase domain of their partner Hsp70 occurs through the highly conserved J domain. To ascertain whether Mpp11 functioned as a J protein in yeast, we exploited the fact that within the J domain of all known J proteins, there is a histidine, proline, aspartic acid tripeptide (HPD motif) that is critical for this interaction (14). We constructed a *MPP11* mutant, $MPP11_{H-Q}$,

that replaced the histidine of its HPD motif with glutamine, an alteration that has been shown to inactivate many J proteins by rendering them incapable of functioning with Hsp70 (15, 16). As expected, $Mpp11_{H-Q}$ was unable to rescue the cation hypersensitivity of a *Δzuo* strain (Fig. 2A and fig. S2).

Because J proteins function only in partnership with an Hsp70, we sought to establish the yeast Hsp70 partner of Mpp11. Ssb is the endogenous Hsp70 partner of Zuo (17, 18). However, no known orthologs of Ssb exist outside of fungi (19). Zuo and Ssb have co-evolved to form a specialized Hsp70:J protein ribosome-associated pair (4). Ssa, on the other hand, is homologous to the abundant, soluble cytosolic Hsc70 of higher eukaryotes. We predicted that human Mpp11 may function in the yeast cytosol by partnering with Ssa, rather than Ssb. To test this hypothesis, we first expressed Mpp11 in a strain lacking Zuo and Ssb, which has the same phenotype as a strain lacking only Zuo (consistent with the evidence that activity of this type of chaperone machinery requires both a J protein and an Hsp70) (4). Mpp11 rescued cells lacking Ssb and Zuo (Fig. 3A) as well as cells

lacking only Zuo (Fig. 2A). Thus, Mpp11 does not require Ssb as an Hsp70 partner to function in yeast.

Ssa is an essential Hsp70. Thus, we could not directly test whether it is required for Mpp11 to functionally substitute for Zuo (20). Instead, we asked whether the ability of Mpp11 to function in yeast was enhanced by increased expression of Ssa. As expected, the presence of a plasmid expressing *SSA1* had no effect on the ability of cells lacking Zuo and Ssb to grow in the presence of cations. However, when plasmids expressing both Mpp11 and *SSA1* were present, growth was improved compared with the strain expressing only Mpp11 (Fig. 3B). This enhancement required a func-
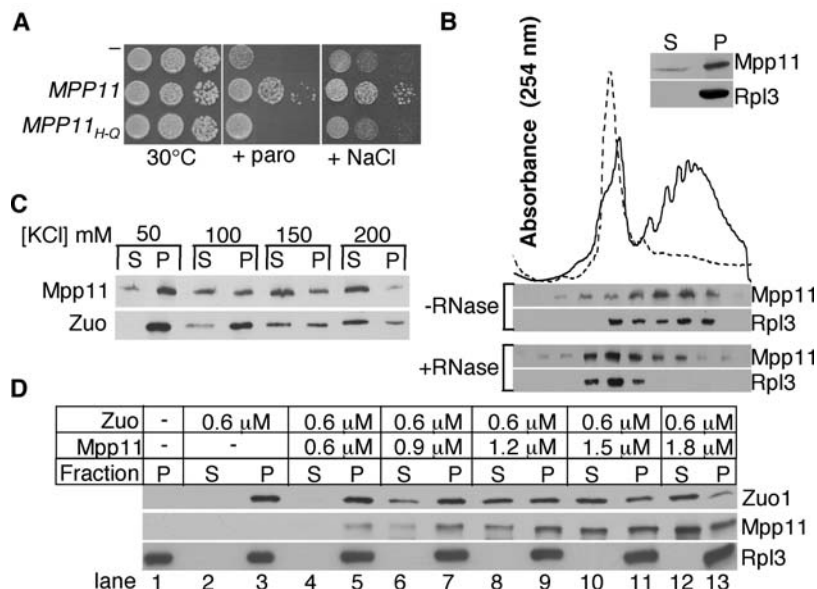
Fig. 3. Mpp11 substitutes for Zuo in the absence of Ssb. (**A**) Yeast strain HH6 expressing *MPP11* was diluted in a 10-fold series, spotted on minimal media and incubated at 30°C (3 days) in the presence and absence of paromomycin (paro) (250 μg/ml). (**B**) HH6 was transformed with control vector plasmids and plasmids expressing *MPP11*, *SSA1*, and both the *SSA1* and either *MPP11* or $MPP11_{H-Q}$. The resulting strains were diluted in a 10-fold series and incubated at 30°C (3 days) in the presence and absence of paromomycin (150 μg/ml). (**C**) Hsp70:ATP-[^{32}P] complexes were incubated in the presence of various concentrations of wild-type Mpp11 or $Mpp11_{H-Q}$ and the rate of hydrolysis of ATP determined. Fold stimulation over the basal rate is plotted. (Left) Mpp11 and Ssa (solid triangles) or Ssb (solid diamonds); $Mpp11_{H-Q}$ and Ssa (open triangles). (Right) Hsc70 and Mpp11 (solid squares) or $Mpp11_{H-Q}$ (open squares).

Fig. 2. Characterization of Mpp11 function in yeast cells lacking Zuo. (**A**) Serial dilution of *Δzuo* cells expressing Mpp11 or $Mpp11_{H-Q}$, which were spotted on minimal media in the presence or absence of paromomycin (paro) (250 μg/ml) and incubated at 30°C (3 days) or 1 M NaCl (5 days). (**B**) (Inset) Lysate of *Δzuo* cells expressing Mpp11-HA was centrifuged through a sucrose cushion separating the ribosome-containing pellet (P) from the supernatant (S). Equivalent amounts of each fraction were subjected to electrophoresis and immunoblot analysis with hemagglutinin (HA)– or Rpl3-specific antibodies. The main panel shows the resuspended pellet, which was incubated in the presence (+) or absence (–) of RNase and centrifuged through a sucrose gradient of 5 to 47%. Optical density (OD_{254}) was monitored (top); fractions were collected and subjected to immunoblot analysis as described above (bottom). (**C**) Ribosome-containing pellets of *Δzuo* cells expressing Mpp11-HA (top) or wild-type cells (bottom) were incubated in the presence of the indicated concentrations of KCl for 30 min at 30°C and then centrifuged through sucrose cushions containing the same salt concentration. Equivalent amounts of the supernatant (S) and pellet (P) fractions were subjected to electrophoresis and immunoblot analysis with HA- or Zuo-specific antibodies. (**D**) Indicated concentrations of purified Zuo and Mpp11 were incubated in the presence of high salt–washed ribosomes (0.6 μM). Samples were centrifuged and the supernatant (S) and pellet (P) fractions were analyzed as described above with the use of the indicated antibodies.
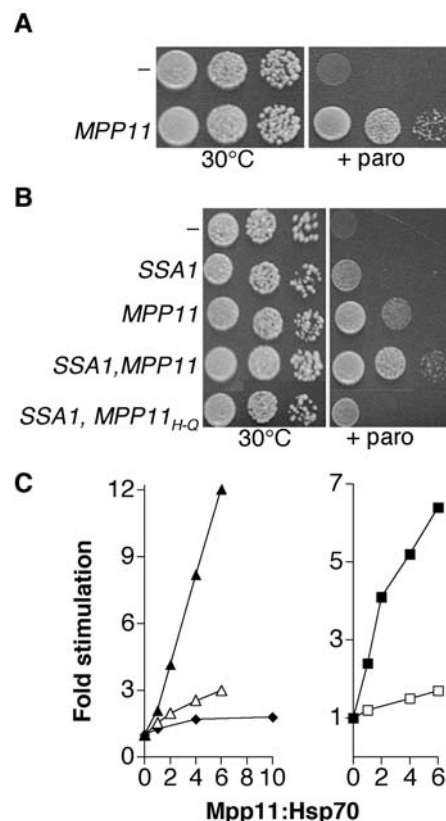
tional J domain, because no suppression was observed when the mutant Mpp11$_{H-Q}$ rather than wild-type Mpp11 was expressed.

The in vivo results described above suggest that Mpp11 functions as the J-protein partner of Ssa, not Ssb. To test this idea biochemically, we asked if Mpp11 could stimulate the ATPase activity of Ssa. Such stimulation, the result of interaction of the J domain with Hsp70's ATPase domain, converts Hsp70 to the adenosine 5′-diphosphate (ADP)–bound form that has high affinity for substrate polypeptides (6, 7). A complex between Hsp70 and radiolabeled ATP was isolated, and the rate of ADP formation was determined upon incubation in the presence of increasing concentration of Mpp11. Mpp11 stimulated the ATPase activity of Ssa 12-fold under the conditions tested, whereas the ability of Mpp11$_{H-Q}$ was severely compromised, as expected of an inactive J protein (Fig. 3C). In contrast, Mpp11 did not substantially stimulate the ATPase activity of Ssb, even when present at a 10-fold molar excess. Thus Mpp11 acts as a J-protein partner of Ssa. If in metazoans, Mpp11 partners with the Ssa homolog, Hsc70, Mpp11 should efficiently stimulate the ATPase activity of human Hsc70. Indeed, Mpp11 stimulated Hsc70's ATPase activity (Fig. 3C). Additional support for such a partnership comes from the identification of *Drosophila* Zuo/Mpp11 ortholog as an interaction partner with *Drosophila* Hsc70 in a genome-wide two-hybrid study (21).

We conclude that Mpp11, an ortholog of yeast Zuo, is a human ribosome-associated J protein. We propose that in metazoans, ribosome-associated Zuo and Mpp11 orthologs recruit the multifunctional soluble cytosolic Hsc70 to short ribosome-bound nascent chains as they exit the ribosome. Consistent with this idea, Hsc70 is not only associated with newly synthesized polypeptide chains recently released from ribosomes, but also with ribosome-associated chains (22, 23). Such recruitment is reminiscent of other cases in which particular J proteins determine the site of action of Hsp70s (24, 25).

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Single, Rapid Coastal Settlement of Asia Revealed by Analysis of Complete Mitochondrial Genomes

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A recent dispersal of modern humans out of Africa is now widely accepted, but the routes taken across Eurasia are still disputed. We show that mitochondrial DNA variation in isolated "relict" populations in southeast Asia supports the view that there was only a single dispersal from Africa, most likely via a southern coastal route, through India and onward into southeast Asia and Australasia. There was an early offshoot, leading ultimately to the settlement of the Near East and Europe, but the main dispersal from India to Australia ~65,000 years ago was rapid, most likely taking only a few thousand years.

The traditional "out of Africa" model for modern human origins posits an ancestry in sub-Saharan Africa, followed by a dispersal via the Levant ~45,000 years ago (1, 2). However, the suggestion of an earlier "southern route" dispersal from the Horn of Africa ~60,000 to 75,000 years ago, along the tropical coast of the Indian Ocean to southeast Asia and Australasia (3, 4), has recently gained ground (5–8). Part of its rationale has been the presence of a number of "relict" populations in southern India and southeast Asia; it has been suggested that these populations might be the descendants of such an earlier dispersal, along with Papuans and Aboriginal Australians (9).

Following the work of Vigilant et al. (10), Watson et al. (11) provided evidence from mitochondrial DNA (mtDNA) patterns for a single dispersal from Africa, although not distinguishing between a northern or southern route. More recently, the existence of a southern route has been supported by analyses of mtDNA restriction enzyme data from New Guinea (12) and control region sequences from main-

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