Multiple elements in the eIF4G1 N-terminus promote assembly of eIF4G1•PABP mRNPs in vivo

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1st Editorial Decision 29 September 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. After some delay due to difficulties with the availability of suitable and willing referees during the past summer holiday period it has now been seen by three referees whose comments are shown below. As you will see while referee 3 feels strongly that the manuscript would be better suited to publication in a more specialised journal the other two referees are considerably more positive and would support publication here after appropriate revision. On balance and given the strong support by the majority of the referees I have decided to invite you to prepare a revised manuscript in which you need to address the issues raised by the referees in an adequate manner. Specifically I would like to urge you to make the manuscript more easily accessible to the non-specialist reader and to place your findings into a broader conceptual context by discussing the more general functional implications of your findings, i.e. on the role of mRNA circularisation via the eIF4G-PABP interaction, in considerably more depth.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

When preparing your letter of response to the referees’ comments, please bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your
The eukaryotic initiation factor 4G (eIF4G) is critical for translation initiation and interacts with multiple translation factors to promote the recruitment of 43S pre-initiation complexes. eIF4G interacts with the cytoplasmic cap-binding protein eIF4E and the DEAD-box protein eIF4A to form the eIF4F complex, which is essential for cap-dependent translation initiation. eIF4G also interacts with the cytoplasmic poly(A)-binding protein PABP bound to the mRNA poly(A) tail. This interaction is thought to promote mRNA circularization by bringing together the mRNA 5' and 3' ends, and to stimulate translation. Although there is compelling evidence indicating that mRNAs can circularize, it remains unclear whether they do so in vivo and which interactions contribute to the formation of an mRNA closed loop, as both PABP and eIF4G interact with additional translation factors.

Previously, regions in eIF4G required for PABP, eIF4E and eIF4A binding have been identified. It was shown that PABP and eIF4E binding are both required for translation; however previous studies have investigated the effect of deleting large protein fragments. This study analyzes the specific contribution of the PABP and eIF4E binding motifs to eIF4G function. The authors have found that the PABP-binding motif is not essential and that additional sequences in the eIF4G N-terminal domain can functionally substitute for the PABP-binding region. Furthermore, several regions in this N-terminal domain appear to contribute in a mutually redundant fashion to mRNA activation, rendering them competent to recruit the 43S pre-initiation complexes. Some of these regions appear to be conserved in yeast but not in metazoa.

The experiments are straightforward and the data convincing. The manuscript also provides new information on the interactions required for translation initiation and, more importantly, shows that mRNA circularization mediated by interactions between eIF4G, eIF4E and PABP is not essential for translation. Clearly, the authors cannot rule out the possibility that the circularization nevertheless occurs and is mediated by additional interactions.

However, the presentation of the results section is rather complex and this could make the manuscript somewhat difficult for the casual reader to follow. The authors could simplify the presentation of the results by simplifying the names of the eIF4G mutants and including a table with a description of what substitutions or deletions have been introduced in each case, e.g. what substitutions are present in the -213 mutant or the -459 mutant, what has been deleted in deltaBox3ext relative to deltabox3, etc. Secondly, the authors should eliminate any reference to genetic backgrounds and refer to the protein mutants using the protein name. The yeast gene encoding eIF4G is termed TIF4631, but switching from the protein to the gene name makes the paper extremely difficult to read. For example, instead of referring to "the tif4631-459 mutant" the authors should write "the eIF4G-459 mutant", etc. The discussion is also quite long and could be shortened.

This is an exciting manuscript because it reexamines a model that is well accepted in the protein synthesis community and finds it needs correction. The original groundbreaking work by Sachs on the role of PABP in translational initiation cannot be faulted, but the conclusions drawn from these his experiments are now shown to be partially erroneous. The more detailed analysis in the current
manuscript reveals these shortcomings but also opens many doors for future studies. Clearly the interactions of RNA, eIF4G, eIF4E, and PABP are more complicated than previous models depicted.

It is a very strong validation of the authors’ findings that the effects of all deletions and mutations the N-terminus of eIF4G are demonstrated both in vivo (growth phenotype) and in vitro (co-immunoprecipitation of PABP and eIF4G). Adding the biophysics with purified proteins (Fig. 7) is the icing on the cake. The biophysics is then able to contribute mechanistic insight to the observations, for instance that the RNA1 region directly contributes to the binding of PABP to eIF4G whereas Box 2 and 3 have little or no effect.

There is very little to suggest by way of improvement of the manuscript. There was one point (p. 13) where I had to read a sentence three or four times before I got the authors point: "However, because coimmunoprecipitation of PABP is already so strongly impaired by the -459,-ΔBox3ext double mutation, we also combined -ΔRNA1 with the -213,-459 double mutation, which as shown above maintains a substantial level of eIF4G1•PABP mRNPs.” They might rephrase it a bit.

Overall, the manuscript is very clearly written despite the complexity of the topic and large number of experiments performed.

Referee #3 (Remarks to the Author):

EMBO 28595 Park et al.

In this manuscript, Park et al. report on a biochemical characterization of eIF4G function in yeast. eIF4G is an important scaffolding protein that serves to form activated mRNPs to trigger the formation of preinitiation complexes and interacts with mRNA, PABP and other initiation factors. To study domains required for the interaction of eIF4G with its binding partners, the authors produced and characterized a plethora of mutants that specifically modify the activity of conserved domains of eIF4G, and combine these mutations with known mutations that have been previously shown to affect eIF4G function. Thus, the authors have generated an impressive data set to dissect the activity of eIF4G and its interaction with PABP and characterize the RNA dependence of this interaction. The data presented are of high technical quality and well controlled.

However, while this study clearly provides insights into the architecture of the eIF4G-PABP mRNPs, I am mostly concerned about the general importance of the study for a broad readership. In my opinion, the authors fail to identify an interesting novel insight that would be of general interest and warrants publication in EMBO. Accordingly, the discussion of the manuscript mostly focuses on carefully explaining the interactions observed in this study, but fails to pinpoint important implications eg. for Ribosome scanning during translation initiation. Therefore, the manuscript seems better suited for a more specialized journal.

Minor points:

1) Overall, due to the multitude of mutants tested, the figures of the paper appear very repetitive. For example, Figures 4A-C, 4 D-F, 5 and 6 present identical experiments with different mutants, with some of the mutants used as control in multiple panels. I wonder if condensing these figures into fewer, but more focused panels might benefit the overall readability. This is perhaps most evident for figure 4.

2) Similarly, Figure 6A in part just summarizes the phenotype of mutations characterized in previous figures. For example -459, ΔBox3ext from Figure 4C, -459,ΔN300 from Figure 1. Instead, it would be more helpful to include the constructs relevant for the IPs presented in Figure 6, in the same order as used for the IP experiments. Accordingly, the -213 mutation should also be included in the schematic drawing in Figure 6A.
Responses to Referees’ comments

We wish to thank the referees for their excellent suggestions, which have helped to us to improve substantially the presentation of our findings.

Referee #1

It was stated that the presentation of results is too complex and should be simplified and that the Discussion was too lengthy. In response, we simplified the names of the mutant alleles, depicted all of the mutants schematically in Fig. 1A, and now always refer exclusively to eIF4G, versus Tif4631, mutant proteins. We shortened the discussion by a whole page, eliminating detailed re-statements of specific results and focusing on the major conclusions and interpretations.

Referee #2

We revised the sentence cited in the third paragraph of the review to make its meaning more transparent.

Referee #3

The reviewer states that we failed “to identify an interesting novel insight that would be of general interest”. In response, we attempted to emphasize more effectively our finding that PABP-eIF4G interaction is dispensable for translation initiation in yeast, even when the cap/eIF4E interaction with eIF4G is eliminated, and that direct interaction of the eIF4G N-terminus with mRNA can functionally substitute for the eIF4G-PABP interaction. These results indicate that mRNA circularization per se via PABP-eIF4G interaction is not critical for initiation, and that the PABP-eIF4G association likely represents only one of several interactions that stabilize eIF4G binding to mRNA, which is the key event required for 40S attachment and scanning. Given the pervasiveness of the closed-loop concept in current models of translation initiation and its regulation by proteins or miRNAs, we think our findings are very significant and of considerable general interest.

Minor points:

1) We eliminated some of the repetition by combining Fig. 4A-C with Figure 3, and Fig. 4D-F with Fig. 5, thus eliminating one whole figure.

2) We also revised this figure (now Fig. 5) to eliminate repetition of mutant constructs from previous figures and to make the schematics of eIF4G1 constructs in the first panel match perfectly with the order of presentation of coimmunoprecipitation data in the subsequent panels. The -213 mutation was added to the schematics.

2nd Editorial Decision

Thank you for sending us your revised manuscript. I have now had a chance to look at your point-by-point response and the new version of the manuscript, and you will be pleased to learn that the paper will now be publishable in The EMBO Journal. You will receive a formal acceptance letter shortly.

Thank you very much again for considering our journal for publication of your work.

Yours sincerely,

Editor
The EMBO Journal