Molecular Cell, Volume *26*

Supplemental Data

The Eukaryotic Translation Initiation Factors eIF1 and eIF1A Induce an Open Conformation of the 40S Ribosome

Lori A. Passmore, T. Martin Schmeing, David Maag, Drew J. Applefield, Michael G. Acker, Mikkel A. Algire, Jon R. Lorsch, and V. Ramakrishnan

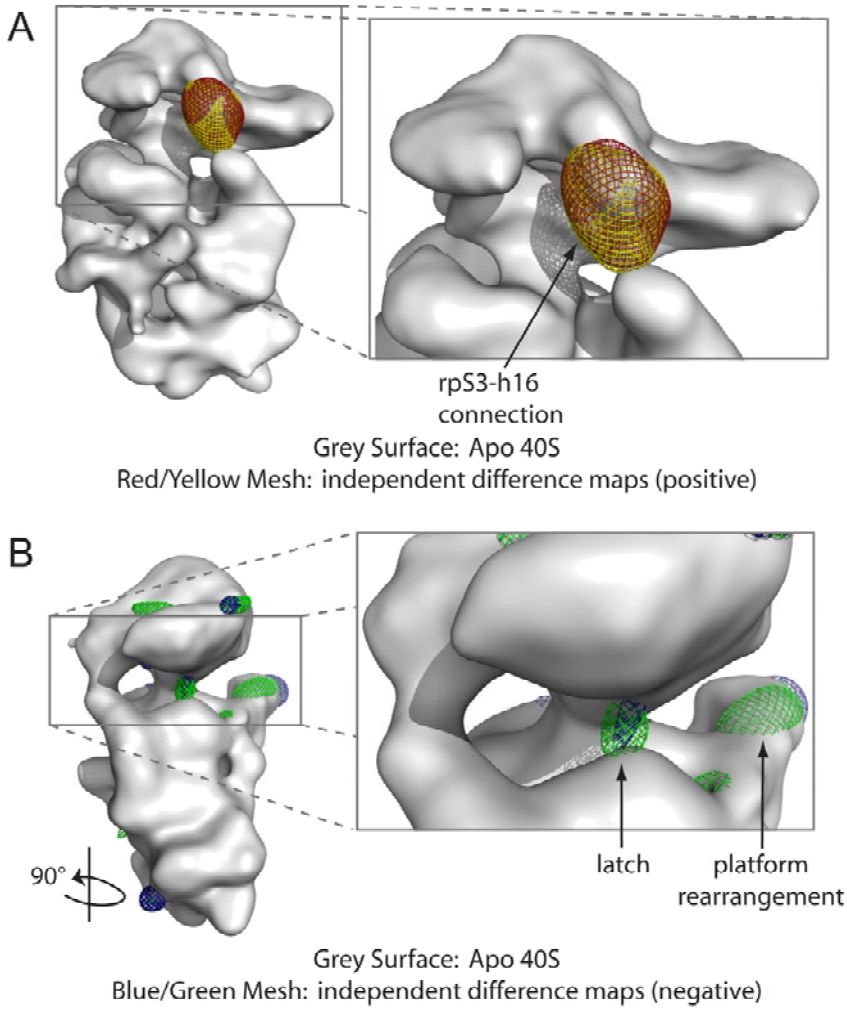

Figure S1: Correlation of two independent difference density maps.

Apo 40S and 40S-eIF1-eIF1A datasets were each split into two equal halves (containing even or odd particles). An independent reconstruction was calculated for each of the four

datasets using an averaged structure of apo 40S and 40S-eIF1-eIF1A (filtered to 20 Å) as a starting model for refinement. Since all four datasets were treated in exactly the same way and refined against a common model, each map is independent. Thus, there is no risk of creating differences between apo 40S and 40S-eIF1-eIF1A structures derived from model bias. Two difference maps were calculated (40S-eIF1-eIF1A$_{map1}$ - apo 40S$_{map1}$ and 40S-eIF1-eIF1A$_{map2}$ - apo 40S$_{map2}$). The two difference maps were overlaid and represented as mesh surfaces. (**A**) Positive difference densities contoured at 22 sigma (red and yellow) and apo 40S (grey surface). (**B**) Negative difference densities contoured -11 sigma (blue and green) and 40S-eIF1-eIF1A (grey surface).

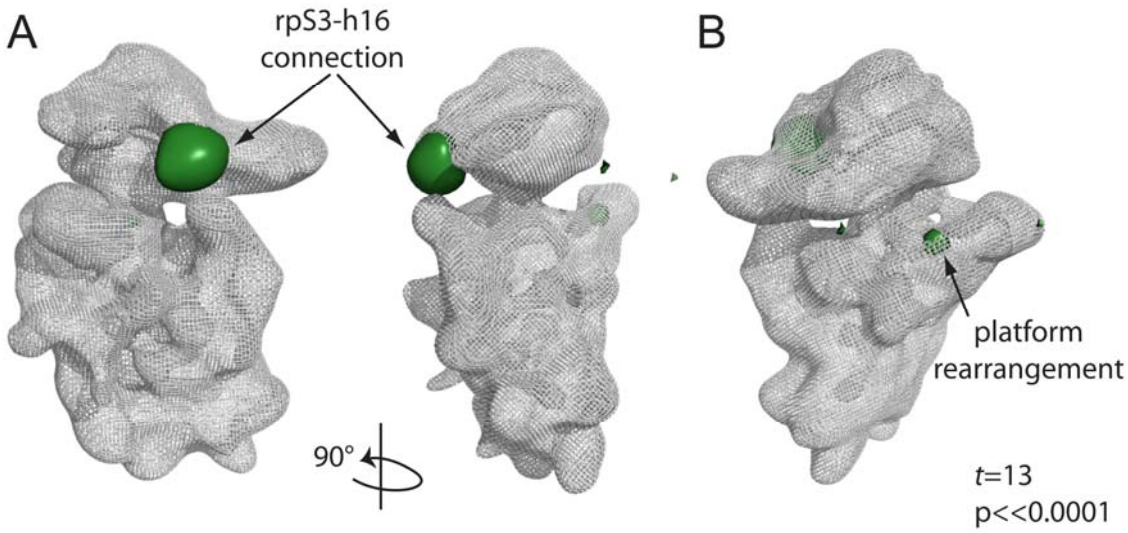

Figure S2: Student's *t* test between apo 40S and 40S-eIF1-eIF1A maps using variance maps calculated by splitting the datasets into four.

To determine the statistical significance of the conformational changes we observed, we performed a Student's *t* test. Apo 40S and 40S-eIF1-eIF1A datasets were each split into four equal parts. Each of the eight datasets was then used to independently refine an averaged structure of apo 40S and 40S-eIF1-eIF1A (filtered to 20 Å) for four cycles. Since all of the datasets were treated in exactly the same way and refined against a common model, each map is independent. Thus, there is no risk of creating differences between apo 40S and 40S-eIF1-eIF1A structures derived from model bias. A Student's *t* test was performed between apo 40S and 40S-eIF1-eIF1A using the programs described by Milligan and Flicker (1987). The 3D *t* map (green) was contoured at t=13 ($p<<0.0001$) and therefore shows only highly significant differences. The *t* map is overlaid on (**A**) apo 40S and (**B**) 40S-eIF1-eIF1A (grey mesh). Because there only ~7500 particles in each dataset, the final resolution of each structure was lower than the resolution of the structures refined with the entire dataset. Thus, the mRNA entry channel latch was not clearly resolved in the apo 40S structure and it is not a significant difference at t=13.

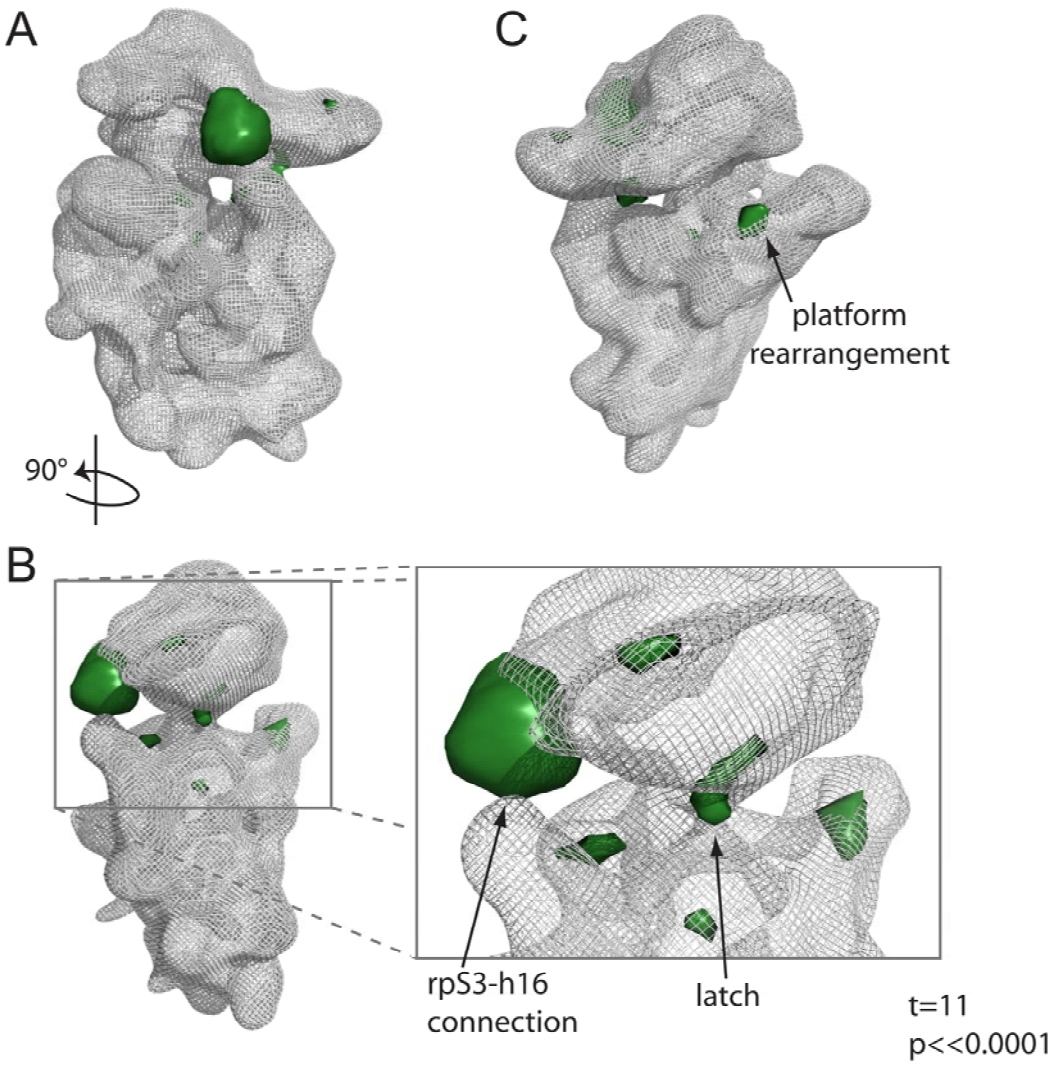

Figure S3: Student's *t* test between apo 40S and 40S-eIF1-eIF1A maps using variance maps calculated with the bootstrap technique. To determine the statistical significance of the conformational changes we observed, we performed a Student's *t* test. We estimated the variance in each reconstruction using a method similar to the bootstrap technique proposed by Penczek et al (2006). Briefly, 100 models each of apo 40S and 40S-eIF1-eIF1A were calculated, each from a random half of the particle set, and these were used to calculated average and variance maps (EMAN programs kindly provided by Junjie Zhang and Steve Ludtke, unpublished). A Student's *t* test was performed between apo 40S and 40S-eIF1-eIF1A using the programs described by Milligan and Flicker (1987). The 3D *t* map (green) was contoured at *t*=11 (p<<0.0001) and therefore shows

only highly significant differences. The *t* map is overlaid on (**A, B**) apo 40S and (**C**) 40S-eIF1-eIF1A (grey mesh).

Supplemental References

Milligan, R.A. and Flicker P.F. (1987). Structural relationships of actin, myosin and tropomyosin revealed by cryo-electron microscopy. J Cell Biol 105:29-39.

Penczek, P.A., Yang, C., Frank, J. and Spahn, C.M.T. (2006). Estimation of variance in single-particle reconstruction using the bootstrap technique. J Struct Biol 154:168-183.