6th March 2014

Your manuscript entitled "Taura syndrome virus IRES initiates translation by binding its tRNA-mRNA-like structural element in the ribosomal decoding center" has now been seen by three referees, whose comments are attached below. In light of their advice we have decided that we cannot offer to publish your manuscript in Nature.

You will see that, while they find your work of some potential interest, our referees raise concerns about the degree of novel insight and about the strength of the novel conclusions that can be drawn at this stage. We feel that these criticisms are sufficiently important so as to preclude publication of your work in Nature.

Although we regret that we cannot offer to publish your paper in Nature for editorial reasons, it might be appropriate for another journal in the Nature Publishing Group family such as Nature Structural and Molecular Biology. More information about this and other NPG journals can be found at www.nature.com/nature/about/family/index.html. Should you wish to have your paper considered by the editors of another NPG journal, please click on this link:

xxx

Using this link you will be able to transfer your manuscript, including the referees' comments and identities, to the editors of the journal you have chosen. Should you wish to have your manuscript considered at another journal without the referees' reports, you will need to submit the paper as a new submission directly to the journal. All of our journals are editorially independent, and the editors of the chosen journal will make their own decision whether to send your manuscript for review.

I am sorry that we cannot be more positive on this occasion but hope that you will find our referees' comments helpful when preparing your paper for submission elsewhere.

With kind regards

Senior Editor
Nature

Referees' comments:

Referee #1:

Koh et al. describe the cryo-EM reconstruction of two structures of S. cerevisiae 80S ribosome bound to the Taura syndrome virus (TSV) IRES at ~6 Å. The TSV IRES belongs to a family of dicistrovirus IRESs that can direct translation without the need of initiation factors. Specifically, the IRESs can be classified into Aparavirus-family and Cripavirus-family IRESs, containing distinct structures. Although extensive studies have focused on the Cripavirus-family IRESs such as CrPV and PSIV, it is thought that despite differences in structure (e.g. SLIII), the Aparavirus-family IRESs function via a similar mechanism, although the function of these distinct structures had not been elucidated. Extensive biochemical and structural studies have pointed to a model whereby the PKI (domain 3) occupies the P site to

initiate translation from a non-AUG codon in the A site. Previous cryo-EM reconstructions of a Cripavirus-family IRES bound to 80S ribosomes could not be definitely resolved with detail to discern the exact location of the PKI domain on the ribosome.

In this study, the authors present a cryo-EM structure that challenges this prevailing model and presents a potentially new model for how this subclass of dicistrovirus IRES initiates translation. Here, the PKI domain of the TSV IRES occupies a density within the A site of the ribosome. Moreover, the study brings to light on the role of SLIII; SLIII is co-axially stacked on the PKI anticodon stem, which protrudes into the large ribosomal subunit to interact with the ASF finger. Their model suggests that the PKI occupies the A site and must be translocated to allow delivery of the first aminoacyl-tRNA. Although this study builds on the existing structural and biochemical data and brings potentially significant mechanistic insights into how these IRESs direct translational initiation, their model would be strengthened with more supportive biochemical evidence.

1) The authors rely heavily on the interpretation of previous biochemical evidence to support their model. Key to this interpretation is the toeprinting results of ribosome/IRES complexes. Previous results showed toeprints of ribosome/IRES complexes at +13-14 (although Pestova and Hellen present evidence of a +14-16 toeprint) given that the C of CCU is +1 in the P site of the ribosome. The interpretation then was that the reverse transcriptase penetrates the IRES/ribosome complex, thus resulting in the +13-14 toeprint. Based on the authors' model, the toeprint at +13-14 is consistent with the PKI domain in the A site. However, the authors do not address the observations of other reported toeprints after the first translocation event in translation extracts or using reconstituted systems. Previous reports have shown that addition of the next two aminoacyl-tRNAs (thus allowing two translocation events) leads to observation of a +6 nt toeprint, which is consistent with the PKI domain
starting in the P site of the ribosome (Pestova, 2003, Genes Dev.; Jan, 2003, PNAS). Based on the authors' model, the toeprint should be +9 nt downstream, which is based on the PKI domain having to translocate first from the A to P sites followed by delivery of the next two aminoacyl-tRNAs. Moreover, the authors do not explain how their model fits with the toeprints in the presence of cycloheximide. Cycloheximide blocks translation elongation by interacting with a deacylated tRNA in the ribosomal E site (Pestova, 2003, Genes Dev.). The IRES allows for two translocation events in the presence of cycloheximide (Wilson, 2000, Cell; Pestova, 2003, Genes Dev.; Jan, 2003, PNAS). The interpretation was that the first aminoacyl-tRNA delivered to the A site has undergo two translocation cycles before reaching the E site of the ribosome where cycloheximide interacts (thus a +6 nt toeprint). However, in the model that the authors present, again, if the PKI domain starts in the A site, then
the toeprint in the presence of cycloheximide would be +9 nt, which is not what has been observed. The interpretation of the toeprinting data is key to their model; without additional supportive biochemical evidence, the authors will need to address this issue.

2) It is clear that from their IRES/ribosome complexes that there is density in the A site of the ribosome, which is potentially very interesting. However, it is not clear if these ribosome/IRES complexes are competent for translation. This is an important control. Also, there is a possibility that the cryo-EM reconstructions may reflect a transient complex, which is trapped during the experimental procedure. For example, the IRES/ribosome complexes were incubated on ice for 5 min prior to flash-freezing. Perhaps the PKI domain is in equilibrium between the P and A sites of the ribosome and becomes trapped under these experimental conditions? Also, it is not clear what buffer is used for the binding reaction. It appears that the TSV IRES RNA was added in excess of ribosomes, why are there only 25% of complexes containing the IRES identified and why are is there so many empty ribosomes identified (or even with a tRNA identified)?

Minor points:

The authors should cite original papers instead of reviews.

For the sentence "This domain is essential for the function of IGR IRESs" Please cite Wilson et al., 2000, Cell; Jan and Sarnow, 2002, JMB; Hatakeyama et al., 2004, RNA.

For the sentence "In agreement with this scheme, translocation of IGR IRESs by one codon...", cite Pestova, 2003, Genes Dev.

Missing key references from:
Hatakeyama et al., 2004, RNA
Sasaki and Nakashima, 2001, PNAS
Kanamori and Nakashima, 2001, RNA

Referee #2:

Korostelev and colleagues report a 6 Angstrom cryo-EM map of the 80S ribosomal initiation complex bound with an intergenic region internal ribosomentry site (IRG IRES) RNA from an eukaryotic virus (TauraSyndrome Virus, TSV).

The structural studies are solid and provide support for the hypothesis of an unprecedented mechanism of translation initiation, which is independent from the canonical, initiator-tRNA-dependent process. The manuscript is clearly written and previous work has been appropriately referenced. The proposed mechanism of IRES-driven translation initiation is certainly of interest, but likely to experts in the field moreso than to a broad readership. Specifically, the cryo-EM structural data at 6 Angstrom resolution is a, certainly more than incremental, improvement of previously obtained structural data for an ribosome complex of an IRES from cricket paralysis virus (CrPV), which was resolved at 20 and 7.3 Angstrom resolution (references 18 and 19). The mechanistic model discussed from pp. 7 on relies predominantly on biochemical data obtained for the CrPV system.

Referee #3:

Cryo-EM at a resolution of ~0.6 nm has been used to obtain structures of the yeast 80S ribosome in complex with TSV IRES. The resulting structures differ from what occurs during canonical initiation, and they demonstrate a conceptually distinct mechanism for positioning the ORF on the ribosome.

The quality of the cryo-EM structure determination is excellent, and until recently it would have been described as being at the cutting edge of the field. While significant biochemical insights could be drawn from these data, I wonder whether the results presented here will soon be superseded by work in which images are obtained using the movie mode of a Falcon 2 (or K2) camera, rather than the Falcon 1 camera used here, and by processing the data with RELION.