Studies of the structure of eRF1•eRF3, eRF1•eRF3•GTP and eRF1•eRF3•GDP complexes.

V.P. Pisareva[1], A.V. Pisarev[1], I.S. Abaeva[1], T.V. Pestova[1] and D.I. Svergun[2]

[1]SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[2] EMBL-Hamburg c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany

Eukaryotic translation termination is mediated by release factors eRF1 and eRF3. The mutual interdependence of eRF1 and eRF3 in termination involves eRF3's stimulation of peptide release induced by eRF1 and stimulation by eRF1 of GTP binding to eRF3 and of eRF3's ribosome-dependent GTPase activity. This interplay between two release factors requires their direct binding through their C-terminal domains. The structures of the three domains of eRF3 resemble those of EF-Tu and eEF1A, but the orientation of eRF3's G domain is different, and switch II is disordered. We hypothesized that binding of eRF1 to eRF3 may influence the position of domain 1 relative to domains 2 and 3 in eRF3 and induce a rearrangement of domain 1 that leads to ordering of the conformation of switch I and switch II such that they better coordinate Mg^{2+} thereby stabilizing its binding to eRF3. Such potential stabilization of Mg^{2+} binding could form the basis for the stimulation by eRF1 of GTP binding to eRF3 and of eRF3's ribosome-dependent GTPase activity. eRF1 could also undergo a series of conformational changes during termination. First, eRF1 has at some point (possibly upon binding to eRF3) to adopt a more closed conformation to fit correctly into the ribosomal binding pocket, because the distance between decoding and peptidyl transferase centers (73Å) is smaller than the distance between the NIKS and GGQ motifs of crystallized eRF1 (98Å)). Second, the eRF1 GGQ motif must be accommodated in the peptidyl transferase center of the 60S subunit, which only occurs after hydrolysis of eRF3-bound GTP.

To characterize aspects of the interaction of eRF1 and eRF3 that contribute to their reciprocal activation, preliminary SAXS experiments were performed on the ribosomal release factors eRF1, eRF3 and their complex [1]. The processed SAXS patterns from these constructs are displayed in Figure 1A. Both eRF1 and eRF3 are dimeric in solution, whereas their complex appears to be largely monomeric. A tentative model of the complex (insert in Figure 1A) was constructed by rigid body refinement employing the available high resolution models of eRF1 and eRF3 in the crystal (PDB entries 1dt9 and 1r5b, respectively). Further experiments will be performed at optimized solvent composition (in particular, by avoiding glycerol at the expense of a somewhat higher salt concentration) to obtain more accurate data for the modelling.

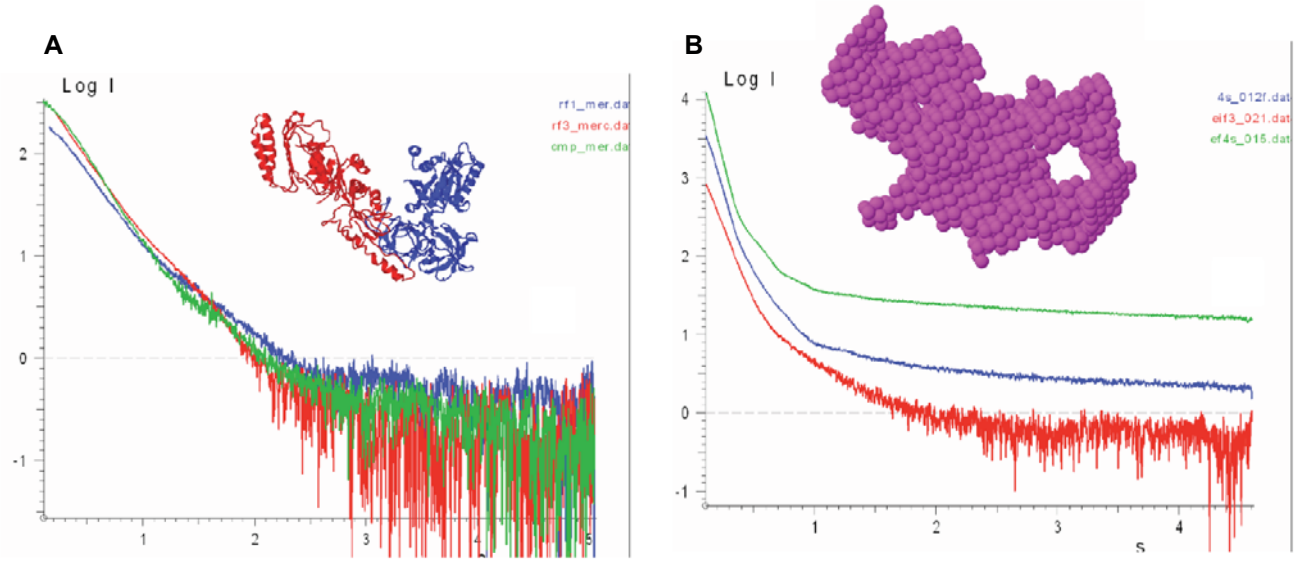

Figure 1. SAXS data and tentative models. (**A**) scattering from eRF1, eRF3 and and their complex (insert: a tentative rigid body model of the complex of eRF1 (red) and /eRF3 (blue)). (B) scattering from the 40S ribosomal subunit, eIF3 protein and their complex (insert: an ab initio bead model of eIF3, bead radius 0.7 nm).

Small-angle X-ray scattering (SAXS) experiments were also performed on solutions of the mammalian translation initiation factor eIF3, 40S eukaryotic ribosomal subunits and their complex [1].

eIF3 is the largest initiation factor that binds many other factors and plays crucial roles at stages in initiation from ribosomal subunit anti-association to 48S complex formation. eIF3 is essential for stimulating binding of eIF2-ternary complex to 40S subunits and ribosomal scanning and may also be directly involved in forming the mRNA-binding cleft on the 40S subunit. The exact mechanism of its activities remains unknown: eIF3 could act directly or indirectly, by changing the 40S subunit's conformation.

The scattering data from all three constructs were collected in the concentration range from 1 to 10 mg/ml and processed using standard procedures (Figure 1B). A tentative low resolution model of eIF3 was constructed from the corresponding scattering pattern (Figure 1B, insert). An attempt was made to localize eIF3 in the initiation complex of eIF3 with 40S. The modelling proved however to be not trivial as the 40S ribosomal subunits apparently tend to form dimers and possibly also higher order oligomers in solution at the concentrations used. Further data analysis is now in progress.

References

[1] Svergun, D. I. and Pestova, T.V. X-ray and neutron small-angle scattering for structure analysis of macromolecular solutions. 7th HFSP Awardees Meeting, Brisbane (2007)