

**P019** Structural and functional characterization of ribosomal translation initiation complexes

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Unlike its prokaryotic counterpart, the small (40S) subunit of the eukaryotic ribosome does not locate directly to the position of the mRNA AUG codon where protein synthesis begins. Instead, it is initially recruited to the capped 5' end of the mRNA, from which point it scans 5'→3' along the 5'untranslated region in search of a start codon. The eukaryotic 40S subunit alone is incapable of assembly onto the capped 5'end of cellular mRNA. Its role in translation initiation depends upon a large number of eukaryotic initiation factors. The 40S ribosomal subunit is prepared for the recruitment step by interaction with a group of eukaryotic initiation factors (eIFs) that, at least in budding yeast, forms a multifactor complex (MFC).

We have applied Atomic Force Microscopy (AFM) to the task of characterising the structural status of the *S.cerevisiae* 40S subunit as it becomes prepared for mRNA recruitment through association with initiation factors. Amino-terminus of the RPS 20 protein of the small ribosomal subunit was modified with GST fusion to immobilize the 40S subunit on glutathione-coated coverslips. RPS 20 is located at the solvent side of the head of the 40S subunit. Functional activity of the GST-40S, as well as the purified eIFs (1, 1A, 2, 3 and 5), was assessed by the ability of the complexes to catalyse methionyl-puromycin formation, when the remaining components required for the assembly of the 80S complex were added. Images of immobilised GST-40S as well as in complex with eIFs were taken in AFM tapping mode. The images reveal structural differences between initiation complexes in comparison with GST-40S.

Key words: ribosome, translation initiation, atomic force microscopy.