

TRANSCRIPTION ELONGATION FACTOR SPT4/5 MODULATES RNA-BINDING OF RNAP SUBUNITS F/E (RPB4/7)

Angela Hirtreiter and Finn Werner, UCL Department of Biochemistry, Darwin Building, Gower Street, London WC1E 6BT, UK

Archaeal RNA Polymerases mirror eukaryotic RNAPII in architecture and use of basal transcription factors. The archaeal RNAP subunits F/E (RPB4/7) form a module that reversibly associates with RNAP and is involved in both initiation and elongation of transcription. During initiation F/E is thought to close the RNAP clamp domain over the DNA binding channel leading to 'open complex' formation (promotor melting). During the elongation phase F/E binds the emerging nascent RNA via an S1 motif (in E) and this interaction has the potential to regulate the processivity and elongation rate of RNAP.

Spt4/5 is a positive transcription elongation factor that is conserved in all domains of life: as heterodimeric complex in eukaryotes and archaea (Spt4/5) and as monomer in bacteria (NusG, homologous to Spt5). Both Spt4 and 5 harbour putative RNA binding motifs, however whether Spt4/5 can bind RNA and how this affects transcription is not clear. Spt4/5 reversibly associates with elongating RNAPs but the subunits and domains that are involved have not been identified yet. In *Sulfolobus acidocaldarius* Spt4 is fused to the C-terminus of RNAP subunit E suggesting a biologically relevant interaction of Spt4/5 and F/E.

The aim of this project is to characterise the interaction network between Spt4/5, RNAP subunits and the nucleic acid scaffold of the elongation complex. We have fused *M. jannaschii* E and Spt4 similar to *S. acidocaldarius*. The E-Spt4 fusion allows the formation of a stable heterotetrameric complex of RNAP-F/E and Spt4/5. Fusion of Spt4 to E marginally decreases the RNA-binding activity of F/E, but the incorporation of Spt5 into the trimeric F/E-Spt4 complex leads to a dramatic stimulation of RNA-binding. Our results show that the N-terminal domain of Spt5 is necessary for the interaction with Spt4. We are discussing the implications of the interaction between F/E and Spt4/5 for the regulation of transcription elongation.