TBP-TAF complex SL1 directs RNA Polymerase I PIC formation and stabilises UBF at the rDNA promoter

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## SUPPLEMENTARY INFORMATION

### Purification of endogenous hSL1

Delineation of the transcription factors involved in rDNA transcription initiation has been reliant on the stringency of their biochemical separation. Previous fractionation of HeLa cell nuclear extract (NE) identified SL1 and UBF, in addition to the RNA Polymerase I enzyme complex (Learned et al., 1985; Learned et al., 1986). The initial separation of these three components from HeLa cell nuclear extract was achieved by chromatography on a Heparin Agarose column (Comai et al., 1992) and represents a significant purification step in the fractionation of SL1 and, therefore, forms the starting point for the further purification of SL1 as outlined (Supplementary Figure, A). Although the Heparin Agarose SL1 fractions alone did not exhibit any transcriptional activity (data not shown), immunoblotting of these pooled fractions revealed the co-fractionation of both a small fraction of the largest subunit of RNA Pol I (A190) and of UBF (Supplementary Figure D, lane 2). Therefore, after desalting on a Sephadex G25 column, SL1 was applied to a Mono S HR column. A linear salt gradient was applied and the peak of SL1 activity, as determined via a reconstituted transcription assay with the rDNA promoter, Pol I and UBF, eluted at 0.3 M KCl, well before the bulk of the protein (data not shown). Peak-fractions of the Mono S column were pooled and subjected to further chromatography on POROS Heparin. The column was developed with a linear salt gradient and SL1 activity eluted between 0.54 and 0.65 M KCl (Supplementary Figure B, lanes 11 to 14). The SL1

peak fractions were pooled and reapplied to the POROS Heparin, but this time eluted in a single step to concentrate the sample. Subsequently, the concentrated SL1 fraction was subjected to gel filtration chromatography on a Superose 6 column. The peak of SL1 activity eluted at around 440 kDa (Supplementary Figure C, lanes 11 to 14). This highly purified and initiation-competent SL1 is free from UBF and Pol I, as judged by immunoblotting (Supplementary Figure D, lane 7), and was used for the stringent functional characterisation of SL1.

**Supplementary Figure.**

Purification procedure separates SL1 from UBF and RNA Pol I.

**A.** HeLa cell nuclear extract was ammonium sulfate precipitated and dialyzed against 0.2 M KCl. The soluble protein mixture was applied to a Heparin agarose column, as previously described (Comai *et al.*, 1992). Further purification steps are outlined, with columns boxed. Linear gradients and step elutions with KCl molarity (M) are indicated. At each chromatographic step the presence of initiation competent hSL1 was monitored using an *in vitro* transcription assay. rRNA synthesised *in vitro* was analysed by S1 nuclease protection using a radiolabeled oligonucleotide overlapping the transcription start site and transcript levels were visualised by autoradiography

**B.** The extent of the linear KCl gradient used to elute from the POROS Heparin column is shown in the graph together with the protein profile (A280 nm) of the elution. The results of the *in vitro* transcription assay for the flow-through (FT) and for each fraction eluted (lanes 1-20) are shown below.

**C.** Step elution from the Superose 6 column was at 0.35 M KCl. The protein profile (A280 nm) of the elution is shown in the graph, with the results of the *in vitro* transcription assay for each fraction eluted (lanes 1-25) below. Migration of the size standards thyroglobulin

(669), Ferritin (440), catalase (232) and adolase (158) from a previous column calibration run are marked in kDa. The void volume was marked with Blue Dextran (bdx).

**D.** Fractions from each chromatographic step in the purification of SL1 were subjected to immunoblotting with antibodies raised against the largest subunit of Pol I (A190), UBF and subunits of SL1 ($TAF_I63$ and TBP; nuclear extract (NE) (lane 1), Heparin Agarose (lane 2), Sephadex G25 (lane 3), Mono S (lane 4), POROS Heparin (lane 5), secondary POROS Heparin (lane 6) and Superose 6 (lane 7).

Supplementary Figure