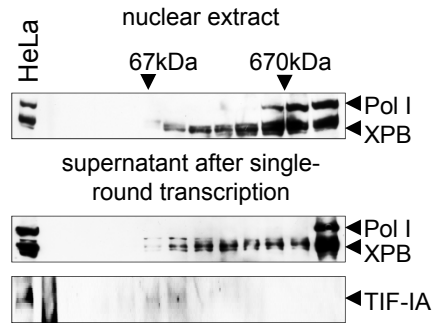


S1



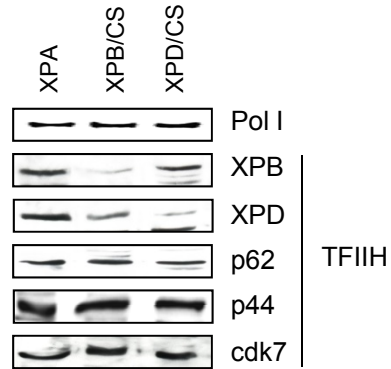
TFIIH sediments with RNA polymerase I after single-round transcription

Nuclear extract or supernatants from single-round transcriptions with immobilized complexes were analysed for sedimentation properties on 12.5-30% glycerol gradients. Whereas TFIIH in nuclear extracts is found in complexes of different sizes, TFIIH sediments after RNA polymerase I transcription in the bottom fraction where RNA polymerase I is detectable. TIF IA sediments after transcription with a molecular size of ca 100kDa.

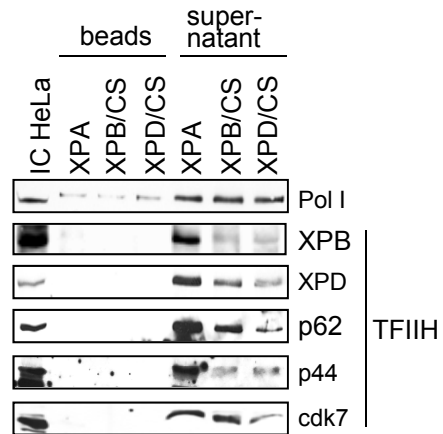
Glycerol-gradient centrifugation

4 ml of a linear 12.5-30% glycerol gradient in buffer AM100 was casted with a gradient maker and overlaid with the indicated probes in 10% glycerol, subject to centrifugation in a Beckman centrifuge and a SW50 rotor for 14h 45000rpm. Twelve fractions were harvested from the top to the bottom and analysed after TCA precipitation on western blots.

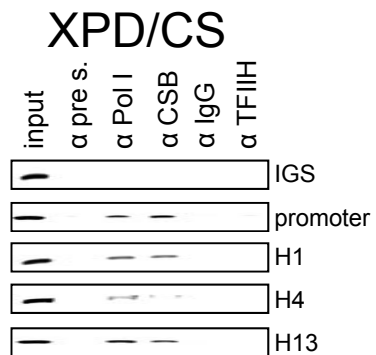
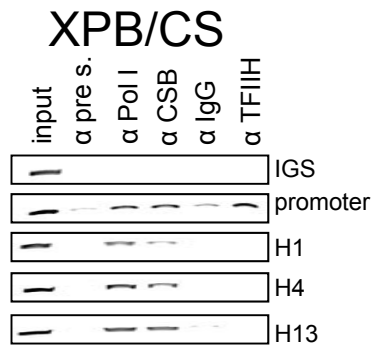
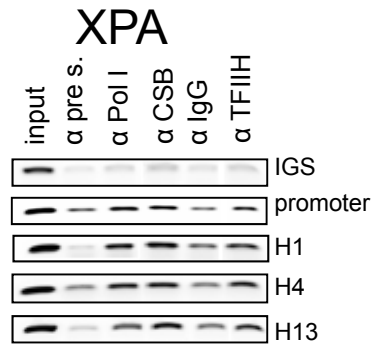
S2



XPB and XPD subunits are reduced in CS nuclear extracts
Western blot analysis of the stoichiometry of TFIIH subunits in nuclear extracts of XPA(wtTFIIH), XPB/CS and XPD/CS cells.

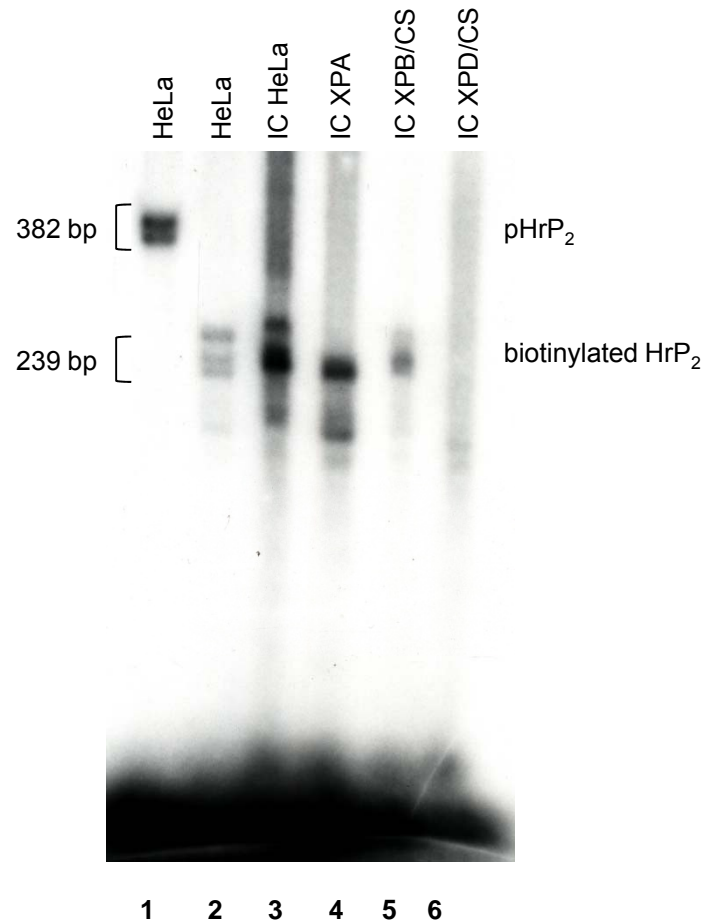


RNA polymerase I does not stall at promoter-proximal sequences when TFIIH is reduced. Western blot analysis of immobilized complexes formed with the indicated nuclear extracts and challenged by the addition of nucleotides. Transcription was restricted to a single round by addition of sarcosyl and unspecific DNA.



CS-mutation in TFIIH is followed by a reduced binding of TFIIH to the rDNA. Semi-quantitative PCR analysis of chromatin-immunoprecipitation experiments performed with the indicated antibodies from chromatin of cell lines as stated above. TFIIH binds the rDNA promoter in XPB/CS cells, but fails to bind to gene-internal sequences. There is no binding of TFIIH in XPD/CS cells to the rDNA detectable

S4



***In vitro* transcription analysis with immobilized complexes does not unravel short or incomplete transcripts.** Picture of the autoradiograph shown in figure 5C. Lane 1. Transcription with HeLa nuclear extract and pHrP₂ template. Lane 2. Transcription with HeLa nuclear extract and biotinylated HrP₂ PCR product as template. Lanes 3-6 single round transcription of immobilized complexes derived of nuclear extracts as stated above.