

Publication

A transcription enhancer acts in vitro over distances of hundreds of basepairs on both circular and linear templates but not on chromatin-reconstituted DNA

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We have analyzed the effect of nucleosome formation and of the simian virus (SV40) enhancer on the efficiency of in vitro transcription. In a whole cell extract made from HeLa cells, nucleosome assembly on DNA results in the formation of chromatin-like complexes. However, transcription was detectable only when the DNA templates were partially or totally depleted of nucleosomes. On nucleosome-free templates, when the SV40 enhancer was present upstream from the complete SV40 early or rabbit beta-globin promoters, there was a five- to tenfold stimulation of specific transcription. When present upstream from its homologous promoter, the SV40 enhancer activated SV40 early transcription independently of its orientation with respect to the coding sequence. Point mutations known to impair the SV40 enhancer function in vivo had a similar effect in vitro. The extent of the enhancing effect was the same with linear or circular templates. When the SV40 enhancer was inserted upstream from the rabbit beta-globin gene, the activation of transcription was reduced with increasing distance between the enhancer and beta-globin upstream promoter elements, but was still significant over a distance of more than 400 base-pairs.

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