

A Basal Transcription Factor That Activates or Represses Transcription
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Supplementary Material

In Vitro Transcription Assay for DSTF/dNC2 Activity

High-salt nuclear extract (1) and low-salt nuclear extract (2) were prepared essentially as described. By Western blot, the low-salt extract has detectable amounts of dNC2, but significantly less than the amount in the high-salt extract. In vitro transcription reactions and primer extension analyses were performed essentially as described (3). Standard reactions for DSTF activity (activation of basal transcription from DPE-driven promoters) were performed with the low-salt nuclear extract, which contains only low levels of endogenous dNC2. Reactions with DSTF/dNC2/Dr1-Drap1 typically contained about 10 to 20 ng of native dNC2 (as estimated by quantitative Western blot with purified recombinant protein as the reference) or about 500 ng of purified recombinant dNC2 (or, as in Web fig. 2, an equimolar amount of each individual subunit), as synthesized either in Sf9 cells with baculovirus vectors or in *Escherichia coli*. The basis for the 25- to 50-fold higher activity of the native dNC2 relative to the recombinant dNC2 is not known. The DNA templates contained minimal core promoter sequences (described below in Supplementary text) subcloned into the Xba I and Pst I sites in the polylinker of pUC119. The amounts of supercoiled DNA template used in each experiment are indicated in the figure legends.

Single-Round Transcription Reactions

Single-round transcription reactions (as in Fig. 4A of main text) were performed as follows. First, preinitiation complexes (PICs) were assembled with template DNA and low-salt nuclear extract (2) in the absence of the four ribonucleoside 5(-triphosphates (rNTPs) for 75 min at 22°C. Second, the rNTPs were added to initiate transcription. Third, Sarkosyl was added (to 0.16% final concentration) 10 s after the addition of the rNTPs. Sarkosyl allows the elongation of transcriptionally engaged polymerase but inhibits transcription reinitiation (6, 7). Lastly, the reaction medium was incubated at 22°C for 45 min to allow completion of the single round of transcription.

References for supplemental material:

1. W. C. Soeller, S. J. Poole, T. Kornberg, *Genes Dev.* 2, 68 (1988).
2. R. T. Kamakaka and J. T. Kadonaga, *Methods Cell Biology* 44, 225 (1994).
3. S. L. Wampler, C. M. Tyree, J. T. Kadonaga, *J. Biol. Chem.* 265, 21223 (1990).
4. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
5. G. M. Rubin et al., *Science* 287, 2222 (2000).
6. D. K. Hawley and R. G. Roeder, *J. Biol. Chem.* 260, 8163 (1985).
7. J. T. Kadonaga, *J. Biol. Chem.* 265, 2624 (1990).