Activation of RNA Polymerase II Transcription by the Specific DNA-binding Protein LSF

INCREASED RATE OF BINDING OF THE BASAL PROMOTER FACTOR TFIIB*

(Received for publication, December 3, 1990, and in revised form, August 23, 1991)

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While the components of the initiation complex at an RNA polymerase II basal promoter have been well characterized, few mechanistic studies have focused on how upstream DNA-binding, transcriptional activators influence protein assembly at the initiation site. Our analysis of basal transcription on both the simian virus 40 and adenovirus major late promoters demonstrates that two slow steps in initiation of transcription are the assembly of the general transcription factors TFIID and TFIIB onto the template DNA. On the simian virus 40 major late promoter, the rate of initiation complex formation is dramatically increased in the presence of the cellular transcriptional activator LSF. Direct analysis by band mobility shift assays demonstrates that LSF has no effect on the rate of binding, or the stability of TFIID on the promoter, predicting that LSF would not affect the template commitment step. Rather, kinetic analyses demonstrate that LSF reduces the lag in the rate of initiation complex formation attributable to the slow addition of TFIIB and suggest that LSF increases the rate of association of TFIIB with the committed template. In addition, LSF increases the total number of transcription complexes in long term assays, which is also consistent with LSF increasing the rate of association of TFIIB, where TFIIB is not saturating. These results indicate a mechanism for the activation of the initiation of RNA polymerase II transcription by one upstream activating protein, LSF. This mechanism may also be applicable to other activators that function in cases where limiting concentrations of TFIIB in the cell dictate slow binding of TFIIB.

Promoter-specific initiation of mRNA synthesis in mamalian cells requires the assembly of RNA polymerase II (RNA pol II)* and multiple additional proteins into an initiation complex at the transcriptional start site of the gene.

*This work was supported by American Cancer Society Grant MV-439 and National Institutes of Health Grant CAG03838 (to U. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: pol II, RNA polymerase II; TF, transcription factor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MLP, major late promoter.

These proteins, termed general transcription factors (TF), have thus far been resolved into the following components: TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIG (1–6). Of these six fractions, only TFIID (also called DB and BTF1) has been shown to bind DNA, specifically recognizing the TATA sequence element found in many RNA pol II promoters (7, 8). Binding of TFIID is the first step in initiation complex formation and commits the template to transcription (9, 10). The subsequent assembly of the remaining factors onto the committed template occurs in at least one defined order (TFIIA, TFIIB, RNA pol II, TFIIE/TFIIF/TFIIG) (11), presumably via protein-protein interactions (reviewed in Ref. 12).

Specific initiation of transcription by the general factors requires only a template consisting of basal promoter elements. The well defined basal adenovirus major late promoter (Ad MLP) consists of a TATA sequence element and an initiator element (13), the latter defined as the initiation site and adjacent sequences. Transcription of the basal promoter by the general transcription factors is distinct from activated transcription, which requires additional DNA sequences and corresponding specific DNA-binding proteins. These activating transcription factors, and the DNA sequences to which they bind, have been the subject of extensive research, due to their key role in the control of initiation of gene-specific transcription. The potential mechanisms by which transcriptional activating proteins might influence the basal transcription machinery, however, are not understood. Previous studies that address this point suggest that either TFIID or, in one instance, TFIIB may be direct targets for the action of particular activating proteins (7, 14–21).

The experiments presented here use a combination of gel band mobility shift analysis and kinetics to determine the mechanism of stimulation by one transcriptional activator, LSF. LSF stimulates transcription from the simian virus 40 (SV40) major late promoter (SV MLP), which directs initiation of the late mode of SV40 transcription from its major in vivo start site, L325. The SV MLP consists of multiple upstream elements and at least one downstream element (22–24 and references therein). The promoter lacks a consensus TATA-box, but does contain a TATA-like sequence which has been shown to be important for late promoter activity in vitro (25). Activation of the initiation of transcription at the SV MLP occurs via LSF binding to a site centered at −45 with respect to the major late start site (24).2 We have now determined that all of the general transcription factors are required for both basal and LSF-activated transcription of the SV MLP. In addition, kinetic analyses revealed the potential rate-limiting steps in complex assembly and demon-

2 M. Shirra and U. Hansen, unpublished observations.
Mechanism of LSF Transcriptional Activation

Preparation of the General Transcription Factors—Nuclear extract (850 mg of protein in 91 ml) was prepared from 100 g of HeLa cells (26) and applied to a phosphocellulose column in buffer C (20 mM Tris-HCl, pH 7.9, 2 mM dithiothreitol, 0.1 mM EDTA, 20% glycerol) plus 0.1 M KCl. The column was sequentially eluted with 0.3 M KCl, 0.5 M KCl, and 1.0 M KCl in buffer C as previously described (27, 28). The phosphocellulose column eluates were further fractionated on DEAE-cellulose columns as described (27, 28), except that the TFIIIE/TFIIF/TFIIG fraction was eluted with a 0.3 M KCl step in buffer C, rather than with a salt gradient. The resulting general factor preparations that also contained SV40 promoter DNA-binding proteins were further fractionated using oligonucleotide-affinity chromatography (29). LSF was removed from the TFIIIE/TFIIF fraction using an LSF-280RS DNA-affinity column, as described (24). AP-1 (30) was removed from the TFIIIE fraction used for Figs. 2, 3, 4A, and 6B by fractionation through a DNA-affinity column containing ligated AP-1 consensus oligonucleotides (a generous gift of C. R. Wobbe and K. Struhl, Harvard Medical School, Boston, MA). The TFIIIE fraction used in Figs. 5 and 6C was purified through two chromatographic steps following the DEAE-cellulose chromatography: a single-stranded DNA-agarose column (27, this material was a generous gift of J. Workman, Massachusetts General Hospital, Boston, MA, and was found to be free of AP-1 DNA binding activity, data not shown), and a DNA-affinity column consisting of ligated copies of the SV40 nucleotide sequences from 318-341, 5'-gtacGACGTACCTAAGCTAG; CCATGGTGCC, which span the HIP1 binding site(s) (31). The DNA-affinity column step was performed in the absence of carrier DNA. TFIIIE eluted at 0.18 M KCl. This latter column separated SV MLP initiator DNA-binding activity (31) from TFIIIE. The level of activation of transcription by LSF was not affected by removal of initiator DNA-binding activity from the TFIIIE fraction (data not shown). The TFIIIE fraction was chromatographed through a second phosphocellulose column to remove contaminating TFIID (Fig. 1d). The TFIIIE/TIIF fraction used for gel band mobility shift analyses (Fig. 4) was further fractionated on heparin-Sepharose as previously described (32). The resulting pools of general transcription factors, dialyzed against buffer C containing 0.1 mM KCl, had the following protein concentrations: TFIIA, 11 mg/ml; TFIIIE (containing HIP1), 5 mg/ml; TFIIIB (lacking HIP1), <0.02 mg/ml; TFIIIE/TFIIIF/TFIIG, 3 mg/ml; TFIID (DEAE-fraction), 0.3 mg/ml; and TFIIID (heparin fraction), <0.1 mg/ml. RNA polymerase II was isolated from calf thymus as previously described (32). The rat factor (TKS phenyl 5-FW fraction) was isolated as described (30) and was a generous gift of J. and R. Conaway. LSF protein was purified from the TFIIIE/TFIIF/TFIIF fraction by successive fractionation over two LSF-280S DNA-affinity columns as described (24). The resulting pool of LSF (3.5 units/μl) was free of all other SV40 specific DNA-binding proteins as assayed by DNase I footprinting (data not shown) (34). One unit is defined as the amount of enzyme required to bind 102 pg of radiolabeled DNA fragment in a band mobility shift assay (Fig. 34).

Plasmid DNA Templates—The plasmid pSVS contains the wild-type SV40 genome cloned into a deletion derivative of pBR322 (36). The mutants of the SV MLP TATA region, pSVS3181, pSVS3182, and pSVS1184, were constructed by site-directed mutagenesis in recombinant M13 phage containing SV40 DNA sequences and then subcloned into the pSVS plasmid background. The mutant pSVS3 was a generous gift of J. Brady (25). The Ad MLP plasmid, pML(CAT)19Δ-50, contains adenovirus MLP sequences from -50 to +10 cloned upstream of the G-less cassette (7). Plasmid DNA templates were purified by using a standard alkaline lysis procedure (37) and were purified by two successive bandings on CsCl density gradients. SV40 viral DNA was obtained from extracts of SV40-infected CV-1 cells (38) and similarly purified.

RESULTS

Transcription of the SV MLP Requires the General Transcription Factors TFIIA, TFIIID, TFIIIE, and TFIIIE/TFIIIF/TFIIF—Promoter-specific RNA pol II transcription can be reconstituted in vitro from partially purified components of a HeLa cell nuclear extract. The chromographic isolation of the necessary components (general transcription factors (TF) TFIIA, TFIIID, TFIIIE, TFIIIF, and TFIIIF) using phosphocellulose and DEAE-cellulose has been described previously (6, 27, 28). Although these transcription factors have been defined as essential for specific initiation of transcription from the Ad MLP, it is thought that they are required by all RNA pol II promoters. Indeed, we previously used all the
partially purified general factors (DEAE-cellulose column fractions) to reconstitute basal transcription of the SV MLP and to demonstrate the activation of that promoter by the transcription factor LSF (24). Further detailed analysis of the mechanism of LSF action required that the general factor transcription system be free of endogenous LSF and other SV40 promoter binding proteins. An examination of the general factors using a DNAse I footprint assay (34) revealed that the partially purified TFIIB was contaminated by both the SV40 promoter binding protein AP-1 (30) and an initiation site binding activity, termed HIP1 (31), and that the TFIIE/TFIIF/TFIIG fraction was contaminated by LSF (data not shown). AP-1, HIP1, and LSF were subsequently removed from the indicated general factor preparations using DNA- affinity chromatography (29). The resulting transcription system retained a high level of transcriptional activity on the Ad MLP, and a minimal basal level of transcriptional activity on the SV MLP, and was responsive to the addition of SV40 transcriptional activating proteins (LSF, this study; and Sp1, Ref. 43, data not shown). Due to the differences between the Ad MLP and the SV MLP, especially in the TATA region (Fig. 1), we were interested in determining whether transcription of the SV MLP, initiated at SV40 nucleotide 925 (SVL325), required in particular the TATA-binding factor TFIIID, as well as all the other general factors.

Transcription of SV40 viral DNA performed with only the general factors resulted in the appearance of a faint, but discrete band representing transcription from SVL325 (Fig. 2, lane 1). In these experiments, addition of LSF to a relatively low concentration (see Fig. 2 legend) led to a 3-fold increase in the level of initiation from SVL325 (lane 7, upper band of doublet). The transcript originating from a downstream start site mapped to SV40 nucleotide position 355 and was also consistently elevated in response to LSF (lane 7, lower band of doublet, marked by arrowhead). This transcript maps close to a minor transcript initiated in vivo (44); its appearance as an abundant product of transcription reactions using the reconstituted system is not fully understood. Omission of any single general transcription factor from the reaction abolished LSF-activated transcription from the SVL325 start site (Fig. 2, lanes 3–6, as compared to lane 2). Surprisingly, initiation at position SVL355 was not absolutely dependent on addition of the TFIIE/TFIIF/TFIIG or TFIIID fractions (lanes 5 and 6). Omission of exogenous RNA pol II from the reaction had little effect on the level of transcription from either SVL325 or SVL355, due to the presence of endogenous HeLa RNA pol II in the TFIIE/TFIIF/TFIIG fraction (data not shown). These results demonstrate that LSF-activated initiation of transcription from the SV MLP requires all of the general transcription factors, including TFIIID. Basal transcription from the SV MLP demonstrated identical requirements (data not shown).

**Mutational Analysis of the SV MLP TATA Region**—The

![Fig. 1. DNA sequences of the SV40 and adenovirus promoters.](Image)

The DNA sequences of the SV40 and Ad MLP basal promoters, from -54 to +13 and -52 to +13, respectively, are shown. The known functional elements within these regions are underlined: the TATA-like sequence, the initiator region, the LSF-280 binding site, the TATA-box, and the initiator element (Inv).
Mechanism of LSF Transcriptional Activation

Fig. 3. Transcriptional analysis of SV MLP TATA-box mutants. A, wild-type and mutant plasmid DNA templates were transcribed in reconstituted reactions containing the complete set of general factors as described in the legend to Fig. 2. The radiolabeled products were analyzed and displayed as described under "Materials and Methods." The preincubation with template was performed either in the absence (lanes 1-5) or presence (lanes 6-10) of 7 units of LSF. The following templates were used: the wild-type promoter, pSVS (lanes 1 and 6), pSVS184 (lanes 2 and 7), pSVS182 (lanes 3 and 8), pSVS3 (lanes 4 and 9), and pSVS3181 (lanes 5 and 10). B, quantitation of the relative transcription activities of the SV MLP TATA-box mutants analyzed in A. The sequences of the wild-type TATA region and each mutant are listed. Levels of transcription from each template were normalized to the basal value on the wild-type promoter.

The absolute levels of LSF-activated transcription on each mutant increased in parallel with the levels of basal transcription (Fig. 3A, lanes 6-10, compared with lanes 1-5), such that the overall fold activation by LSF remained fairly constant, within the range of 12- to 20-fold (Fig. 3B). We note that in these experiments LSF was present during the entire incubation period, in contrast to the kinetic experiments described below, where LSF was added subsequent to the preincubation (Fig. 6). Therefore, increasing the efficiency of the basal promoter, due apparently to the increased binding affinity of TFIIID, had little effect on the ability of LSF to activate transcription.

LSF Has No Effect on the On/Off Rate of TFIIID Binding to the Promoter Sequences—Given the proximity of the LSF binding site relative to the SV MLP TATA-like sequence and previous reports that upstream activating proteins might target TFIIID (7, 14-20), a direct analysis was undertaken of the effect of LSF on binding of TFIIID to DNA. These studies were performed with TFIIID purified from HeLa cells, as bacterially produced human TFIIID will not support activation by LSF in vitro (data not shown). TFIIID purified as described (see "Materials and Methods") bound DNA to form specific complexes that migrated upon nondenaturing gel electrophoresis in positions comparable to those observed by others (see below and Refs. 47 and 48). In addition, the purified HeLa TFIIID produced a characteristic DNase I footprint on the Ad MLP, including protection of the TATA sequences as well as hypersensitive cleavages covering adjacent regions (8) (data not shown).

Incubation of TFIIID and TFIIA with a radiolabeled DNA fragment from the SV MLP pSVS3181 TATA consensus mutant and analysis of the complexes by electrophoresis through nondenaturing gels resulted in a major species (Fig. 4A, lanes 1 and 5) that was efficiently competed by an excess of consensus TATA sequences (3181 TATA, lane 7), minimally competed by SV40 wild-type TATA sequences (wt TATA-like, lane 6), and resistant to competition by an unrelated DNA sequence containing an LSF binding site (GC123, lane 8). Addition to the binding reaction of TFIIB or $\alpha$, the rat TFIIB analogue (33), resulted in the generation of new complexes DAB and DAda (lanes 2 and 3, respectively), which migrate more slowly than the DA complex. The relative migration of the DA and DAda complexes appears similar to that demonstrated previously using HeLa cell factors and the Ad MLP (48).

The LSF-DNA complex migrated slightly slower than the DA complex (Fig. 4A, lanes 13-16, compared to lane 5) and was not competed by either the consensus 3181 TATA or wt TATA-like DNAs (Fig. 4A, lanes 14 and 15), but was efficiently competed by the GC123 DNA (Fig. 4A, lane 16), as expected (24). Upon co-incubation of TFIIA, TFIIID, and LSF with template DNA, a new, more slowly migrating complex was observed (Fig. 4A, lane 9, LSF-DA). The effective competition by both the 3181 TATA DNA (Fig. 4A, lane 11) and the GC123 DNA (Fig. 4A, lane 12), but not the wt TATA-like DNA (Fig. 4A, lane 10), demonstrated that this is a specific complex containing both LSF and TFIIID (and probably TFIIA). Competition by the GC123 DNA drove the LSF-DA complex into the DA complex (Fig. 4A, lane 9' compared to lane 12'), which provides further evidence for the presence of TFIIID (and TFIIA) in the LSF-DA complex. In addition, the amount of competitor GC123 DNA was not sufficient to compete for all of the LSF-DNA complexes (Fig. 4A, lanes 12 and 12'), resulting in a low level of LSF-DNA complex that was resolved from the DA complex (Fig. 4A, lane 12').

The presence of the individual LSF-DNA and TFIIID-TFIIA-DNA complexes in the same reaction in the absence of LSF-DA complexes and the nearly saturating amounts of LSF required to generate the quaternary complex suggest that there is no cooperativity of binding between LSF and TFIIID.

With identification of these complexes, we could test directly whether LSF might activate transcription either by increasing the rate of binding of TFIIID or by decreasing the dissociation rate of bound TFIIID. The effect of LSF on the DNA-binding kinetics of TFIIID are shown in Fig. 4, B and C. The binding of TFIIID alone to the TATA-containing SV40 promoter DNA was slow (Fig. 4B, lanes 1-8; DA complex) and began to plateau at about 90 min. In contrast, the rate of binding of LSF was extremely fast and complete by 5 min (Fig. 4B, lane 10). The effect of bound LSF on a subsequent TFIIID binding event is demonstrated in Fig. 4B, lanes 9-16. Neither the rate nor amount of TFIIID binding to the DNA appeared to be altered by the presence of high levels of LSF. Instead, the rate of accumulation of the LSF-DA-DNA complex paralleled that of the DA-DNA complex.

To test the effect of LSF on the stability of TFIIID-TFIIA-DNA complexes, the rate of dissociation of bound TFIIID-TFIIA was measured, both in the absence and presence of LSF. DA-DNA or LSF-DA-DNA complexes were formed by incubation of the proteins and template DNA for 120 min. Subsequently, the competitor double-stranded oligonucleotide containing the consensus TATA sequence was added in an amount empirically determined to prevent formation of new...
Fig. 4. Gel band mobility shift analysis of effects of LSF on the binding of TFIID. A, demonstration of complex specificity. Protein-DNA binding reactions containing the indicated proteins and competitors were combined in 10-μl reactions as described under “Materials and Methods.” After preincubation at 30°C for 10 min, template DNA was added and the incubation at 30°C was continued for another 30 min before loading onto the gel. Arrows indicate the positions of migration of specific protein-DNA complexes: DA, TFIID-TFIIA-DNA; DAα, TFIID-TFIIA-TFIIA-α-DNA; DAB, TFIID-TFIIA-TFIIB-DNA; LSFD'A, LSF-TFIID-TFIIA-DNA; and LSF, LSF-DNA. An asterisk marks the position of a faster migrating complex formed with the TFIID and TFIIA fractions, which is believed to be formed from a proteolytic product of TFIID.

DA-DNA complexes, but which would not affect complexes that had already formed (data not shown). A time course after the addition of competitor represents the dissociation of TFIID. The rate of dissociation was fairly slow (DA; Fig. 4C, lanes 1–8) and was unaffected by the presence of bound LSF (LSF-D'A; Fig. 4C, lanes 9–16). These data, taken together, demonstrate that LSF has no detectable effect on the specific binding of TFIID to DNA, arguing that TFIID is not the functional target for LSF-mediated activation of transcription.

Kinetics of Initiation of the Adenovirus MLP—A kinetic approach was undertaken both to identify the slow steps involved in initiation complex assembly and to investigate the effects of LSF on these steps. Extensive kinetic analyses of transcription initiation complex assembly on bacterial promoters provide a model from which to initiate experiments on eukaryotic transcription complex assembly. A lag in the approach to a steady state rate of initiation complex assembly has been defined as the average time for Escherichia coli RNA polymerase to form an open-promoter complex (49). The time of the lag can represent one or a combination of slow steps in complex assembly and, under certain conditions, may reflect the rate-limiting step in complex formation. Lag time can be determined experimentally by extrapolation to zero product on a plot of product versus time (49). We have performed a similar analysis on several promoters to define the slow or rate-limiting step(s) in RNA polymerase II initiation.

Previously, the basal RNA pol II transcription factors have been shown to be able to assemble onto promoter-containing DNA in the following order: TFIID-TFIIA, TFIIH, RNA pol II, TFIIIE/TFIIF (10, 11). Alternative pathways for the assembly may also be possible, with RNA pol II addition before or with TFIIB (28). This assembly results in a “rapid-start” complex (50) capable of immediately incorporating nucleoside triphosphates into specifically initiated, nascent RNA. We began a kinetic analysis using the HeLa transcription factors on the well studied Ad MLP to establish whether the approach would distinguish separate steps in complex assembly. The Ad MLP template was initially incubated with different subsets of general factors, based on the sequential assembly indicated above (Fig. 6A). This preincubation was followed by the simultaneous addition of the complement of general factors and radiolabeled nucleotides at low concentrations (Fig. 6A) and a subsequent incubation for short lengths of time. Finally, transcripts were fully elongated during an incubation with high concentrations of unlabelled nucleotides. Complete initiation complexes cannot form unless all of the general factors are present. Therefore, in reactions lacking...
one or more of the factors during the preincubation, the observed level of transcripts reflects the number of rapid-start complexes formed by the different time points, or the rate of completion of initiation complexes, following the addition of omitted factors. Thus, the level of transcription is correlated with the rate of assembly of the rapid-start complex. This is in contrast to the experiments of Fig. 3, where the level of transcription reflected the number of initiation complexes formed during a long preincubation step and correlated with the overall stability of the complex.

When all general factors were preincubated with DNA, there was a rapid and linear transcriptional response to the addition of nucleotides (Fig. 5A, lanes 9–12 and open squares, Fig. 5B). Extrapolation of the curve to zero product at zero time demonstrates no lag in initiation on preformed complexes, as expected (28, 49). When no factors were preincubated with the DNA, there was a significant lag in the rate of initiation complex assembly (Fig. 5A, lanes 13–16 and closed circles, Fig. 5B). Preincubation of the template DNA with TFIIA and TFIIID reduced the lag slightly (Fig. 5A, lanes 1–4 and open circles, Fig. 5B), indicating that the binding of TFIIID is a slow process that can be overcome by prebinding. Additionally, preincubation of TFIIIB substantially affected the linearity of the response (Fig. 5A, lanes 5–8 and closed squares, Fig. 5B), indicating that another slow step had been overcome by the preincubation. These data could be interpreted in two ways: either the binding of TFIIIB is an additional, slow step or TFIIIB stabilizes the TFIIID complexes formed on the DNA during the preincubation. To discriminate between these possibilities, another experiment was performed in which the (the rat analogue of TFIIIB) was either preincubated with TFIIID and TFIIA or added at increasing concentrations subsequent to preincubation with TFIIID and TFIIA and the DNA. The striking observation (data not shown) was that at sufficiently high levels of added with nucleotides, the kinetics of product formation were identical with those observed when was present during the entire incubation. The ability to abolish the slow step with high concentrations of highly purified indicates that rather than stabilizing the TFIIID-DNA complex, the actual binding of is the slow step under normal reaction conditions. Thus, binding of TFIIB under these conditions is slow and may be rate-limiting.

In studies of bacterial promoter initiation kinetics (49), reactions that received no preincubation eventually reached the same steady state rate of product formation as those that had been preincubated, i.e. the curves at late time points became parallel. In the experiments presented here, we have limited the analysis to early time points where the data are the most illuminating and, due to considerations of factor stability, are the most reliable. However, similar to what was observed in the experiments with E. coli RNA polymerase, the curves in Fig. 5B representing reactions that received an incomplete set of factors in the preincubations (closed squares, open circles, closed circles) are becoming parallel with the curve for the reaction that received all factors in the preincubation (Fig. 5B, open squares). Reinitiation, the repeated entry of RNA pol II into a rapid start complex on a single template, may be contributing somewhat to the level of transcripts detected in these assays. However, reinitiation levels would be low, especially at the early pulse time points due to the low concentration of nucleotides added during the pulse labeling stage of the reaction (9) and to the relatively slow rate at which reinitiation takes place (51). The interpretation of these data do not require that the reaction be limited to a single round of initiation. The increase in slope of the curves at later time points is evidence that the reactions are approaching a steady state rate of initiation, i.e. an average of the rates for all events related to production of signal (from formation of the rapid start complex through limited elongation, promoter clearance, and reinitiation). It has been demonstrated that after “promoter clearance” by RNA pol II, the only basal transcription factor detected at the promoter is TFIIID (52). Therefore, in order for reinitiation to occur, the slow TFIIIB binding step must be repeated. This event and subsequent completion of the rapid start complex are thus indistinguishable kinetically from the first round of initiation on that template molecule (in the case where TFIIID is pre-bound).

These results demonstrate that the rate of completion of initiation complexes is sensitive to and depends upon which factors are present in the preincubation step and validates the approach for examining individual steps in RNA pol II initiation complex assembly.

**LSF Increases the Rate of TFIIIB Binding to the Committed Template**—A kinetic analysis was performed on the basal SV MLP in order to investigate the generality of the rate-limiting steps in initiation at basal RNA pol II promoters. In parallel, LSF was added following preincubation of the different sets of factors to determine at which step LSF exerts its effect in the assembly of the rapid-start complex (Fig. 6A). The effect of the strength of the TFIIID binding site on the mechanism of LSF activation was analyzed by comparing the rates of complex assembly on the wild-type SV MLP (in pSVS, Fig. 6B), to those on a mutant SV MLP containing the consensus TATA sequence (in pSVS3181, Fig. 6C).

Experiments performed in the absence of LSF delineated the slow steps in the basal initiation process on the SV40 late promoter. The TATA consensus mutant was transcribed at higher levels after preincubation with TFIIA and TFIIID than after preincubation with no factors (closed circles, Fig. 6C, panel ii versus panel i), consistent with template commitment and preferential transcription due to the binding of TFIIID (10). After preincubation of the TATA consensus mutant with TFIIA, TFIIID, and TFIIIB, there was a significant re-
Mechanism of LSF Transcriptional Activation

**Fig. 6.** Kinetic analysis of initiation complex assembly on the wild-type and consensus TATA containing SV MLPs. A, the order of addition of various components to the transcription reactions is outlined. Template DNAs and various general factors were preincubated prior to the addition of the complement of factors, either 7 units of LSF or control buffer, and radiolabeled nucleotides. 2, 5, 10, or 20 min after the addition of radiolabeled nucleotides, high concentrations of unlabeled nucleotides were added for 2 to 3 min, and reactions were stopped and processed as described under "Materials and Methods." B, stimulation of transcription by LSF on the wild-type SV MLP template, following preincubation with various combinations of factors. Transcription reactions were performed as outlined in A and as described under "Materials and Methods." The preincubation reactions represented in each panel contained DNA and either no general factors (panel i), TFIIA and TFIID (panel ii), TFIIA, TFIID, and TFIIB (panel iii) or all the general factors (panel iv). The accumulation of the SVL325 transcript is plotted versus time of the pulse. Levels of transcription in all panels are normalized to the level at the 2-min time point in panel i (0.1). Given that the experiments were performed with different batches of [α-32P]UTP, the comparison of absolute levels of transcription between panels may not be entirely precise. *Open symbols* represent reactions that received LSF. *Closed symbols* represent reactions that received control buffer. C, stimulation of transcription by LSF on the consensus TATA SV MLP template, following preincubation with various combinations of factors as described in A and B. The accumulation of the SVL325 transcript is plotted versus time of the pulse. There is no *open symbol* at 10 min in panel iii because the curve is extrapolated to the 20-min time point (not shown). Levels of transcript are normalized to the level at the 2-min time point in panel i (0.2). *Open symbols* represent reactions that received LSF. *Closed symbols* represent reactions that received control buffer. The fold activation by LSF at the 10-min time point in each panel is as follows. B: panel i, 8; panel ii, 31; panel iii, 4; panel iv, 2; C: panel i, 36; panel ii, 51; panel iii, 3; panel iv, 2.

production in lag time (closed circles, Fig. 6C, panel iii), compared to preincubation with TFIIA and TFIID alone (closed circles, Fig. 6C, panel ii), which indicates the elimination of a slow step in complex assembly. As argued above for the Ad MLP, this slow step is due to binding of TFIIB. Preincubation with TFIIID and TFIIA resulted in no detectable effect on the rate of basal transcription from the wild-type promoter (closed circles, Fig. 6B, panel ii versus panel i). Presumably, commitment on the wild-type template was not observed because of the 17-fold lower affinity of TFIIID to this promoter. However, as with the TATA consensus mutant promoter, preincubation with TFIIA, TFIIID, and TFIIB on the wild-type promoter (closed circles, Fig. 6B, panel iii) was sufficient to allow substantial accumulation of the TFIIA-TFIID-TFIIB-DNA complexes and to significantly increase the rate of rapid-start complex assembly (greater than 9-fold and 6-fold transcriptional increases on the wild-type and the TATA consensus promoters, respectively, at 5 min; Fig. 6, B and C, panels iii versus panels ii). Thus, the slow assembly rates of basal initiation observed in panels i and ii were due, at least in part, to the slow binding of TFIIB. The rates of basal complex assembly for reactions preincubated with TFIIA, TFIIID, and TFIIB were very similar to rates of assembly observed when all general factors were present in the preincubation (closed circles, Fig. 6B and C, panels iv versus panels ii); no difference on the wild-type
complex assembly on the wild-type promoter and only slightly increased it on the TATA consensus promoter.

When LSF was added along with the complement of general factors and nucleotides (Fig. 6A) to reactions preincubated with no factors (open circles, panel i of Fig. 6B, panel i, and a 10-fold increase on the TATA consensus promoter, Fig. 6C, panel i, at 10 min). The degree to which LSF stimulated transcription varied at every time point in the reaction, as well as from reaction to reaction (see numbers below). Thus, LSF cannot be simply causing an increased rate of initiation from preformed transcription complexes. Instead, LSF must be specifically increasing the rate at which the rapid-start complexes are formed and recruiting new complexes competent for initiation. Given that the basal promoter analysis established that both the TFIIID and TFIIIB assembly steps limit the rate of initiation complex assembly, the rapid activation observed in the presence of LSF indicated that LSF is accelerating the assembly of TFIIID and/or TFIIIB. Preincubation with TFIIA and TFIIID did not affect the LSF-activated rate of initiation complex assembly on the wild-type promoter and only slightly increased it on the TATA consensus promoter (open circles, Fig. 6B, panel i). Since there was little reduction in the lag upon preincubation with TFIIID (open circles, panel ii compared to panel i), it is unlikely that LSF is targeting TFIIID alone.

Two significantly slow steps in the assembly of RNA pol II initiation complexes have been demonstrated: the binding of TFIIID to the DNA and the assembly of TFIIIB onto the TFIIID-TFIIA-DNA complex. By the kinetic analysis described above and by direct binding analysis (Fig. 4), LSF has been shown to have no detectable effect on the binding of TFIIID. Therefore, by the process of elimination, in order to achieve high rates of initiation in the absence of preincubation, LSF must accelerate the subsequent slow step, the binding of TFIIIB, to the TFIIID-TFIIA-DNA complex. Consistent with this, preincubation with TFIIIB significantly reduced the need for LSF in the production of relatively high levels of transcription (only 4-fold activation by LSF in Fig. 6B, panel iii, compared to 30-fold in Fig. 6B, panel ii, at 10 min). Following preincubation with TFIIA, TFIIID, and TFIIIB, the rate of initiation complex assembly on the wild-type promoter in the presence of LSF still demonstrated a substantial lag, whereas on the TATA consensus mutant, the rate was strikingly linear after a minimal lag of 1-2 min (open circles, compare Fig. 6, B and C, panels iii). The long lag on the wild-type promoter presumably reflects the low levels of TFIIA-TFIIID-TFIIIB complexes formed on this promoter during the preincubation and is due to the slow formation, even in the presence of LSF, of the TFIIID-DNA complex. On the TATA consensus promoter, the slow TFIIID binding step (9, 10, 28) has presumably largely been completed, resulting in only a minimal lag. The data are consistent with LSF causing the rapid association of TFIIIB to those relatively stable TFIIA-TFIIID-DNA complexes on the TATA consensus promoter that were formed during the preincubation but that had not yet bound TFIIID (see Fig. 7). Thus, the substantial lag on the wild-type promoter (Fig. 6B, panel iii) again suggests that LSF does not have a stimulatory effect on the rate of binding of TFIIID to the promoter.

Preincubation of both the wild-type and TATA consensus promoters with either all of the general factors (Fig. 6B, panel iv) or with only TFIIA, TFIIID, and TFIIIB (Fig. 6C, panel iii) resulted both in similar extents of activation by LSF and in similar shapes of the curves. This indicates that the major effect of LSF in increasing the rate of initiation complex formation is on steps prior to addition of TFIIIE-TFIIIF/TFIIG and RNA pol II. The minor only differences was that preincubation of the wild-type promoter with all of the general factors (Fig. 6B, panel iv) rendered the reaction relatively unresponsive to the addition of LSF in the first 5 to 10 min of the pulse, compared to the reaction preincubated with TFIIA, TFIIID, and TFIIIB (Fig. 6B, panel iii). However, the reaction in which template was preincubated with all general factors did respond to the addition of LSF upon a 20-min pulse, where a greater than 4-fold increase in transcription was observed over that in the reaction receiving no LSF (Fig. 6B, panel iv). In contrast, the TATA consensus promoter responded similarly to LSF when preincubated either with TFIIA, TFIIID, and TFIIIB (Fig. 6C, panel iii) or with all of the general factors (Fig. 6C, panel iv). The apparent delayed response to LSF on the wild-type promoter may be due to the formation of nonproductive initiation complexes during the preincubation at cryptic TATA sequences on the wild-type SV MLP DNA template. These would be less likely to form on the TATA consensus template, which would instead form productive initiation complexes at the authentic high affinity TFIIID binding site (TATA-box) in the SV MLP sequence of pSVS3181.

**Discussion**

**cis- and trans-Components of the SV MLP**—In view of our demonstration that the general factor requirements of the SV MLP and Ad MLP are similar (Fig. 2), it might be expected that the two basal promoters would have similar functional elements. A comparison of the DNA sequences within the SV MLP and the Ad MLP, however, reveals no striking similarity (Fig. 1). The basal adenovirus promoter consists of a TATA-box to which TFIIID binds (7, 8) and an initiator element (Inr) (13, 53) for which no known binding protein has been found. The sequences upstream of the TATA-box and downstream of the Inr element can be deleted without significantly affecting the level of basal transcription from the promoter. It has been suggested, however, that the G-rich sequences between the TATA-box and the Inr element may influence the efficiency of TATA function, presumably at the level of binding of TFIIID (46, 54).

SV40 late promoter elements have been characterized both in vivo and in vitro and consist of multiple upstream regula-
ory regions (for a discussion see Ref. 24) and at least one downstream element (22, 23). Analyses of SV40 transcription have been performed either in vivo or in crude extracts which contain many SV40 regulatory proteins, thus the distinction between regulatory versus minimal or basal elements has not been made. The TATA-like region of the SV MLP is a presumed basal element (Fig. 1) and was shown to be important for SV40 late transcription in vitro in unfractionated cellular extracts (25). Due to i) the sequence analogy with the Ad MLP, ii) the efficiencies of binding of TFII D to the wild-type SV MLP TATA-like sequence and pSVS3181 TATA sequence in gel mobility shift assays correlating with efficiencies of wild-type SV MLP and pSVS3181 basal transcription, respectively, and iii) the requirement of SV MLP transcription for TFII D (Fig. 2), it can be concluded that the wild-type SV MLP does indeed contain a sequence that is functionally recognized by TFII D. In addition, the SV MLP contains a sequence at the SV40 L325 start site (Fig. 1) that is very similar to the initiator element from the dihydrofolate reductase (DHFR) promoter (31), which appears to operate similarly to the Ad MLP Inr (13) in specifying the start site of transcription. Thus, despite the obvious differences in sequence, the basal adenovirus and SV40 major late promoters appear to be functionally similar.

**Mutational Analysis of the SV MLP TATA-box**—Binding of TFII D to the promoter-template DNA is the first step in initiation complex assembly (9, 11) at the Ad MLP and serves to commit that template to specific transcription (9, 10). It is not known specifically how TFII D exerts its effect on initiation once it is bound to the DNA. The position dependence of the TATA-box suggests that the bound TFII D may serve as a molecular magnet to attract the remaining general factors and RNA polymerase II to the proper positions surrounding the start site of transcription. Since TFII D is the only general factor known to bind DNA specifically and TFII D binding to the TATA-box is the first step in complex formation, TFII D has been considered a likely target for the action of upstream activating proteins. Several studies have suggested that the binding of TFII D in particular may be affected. Cooperative interactions (7) and direct protein-protein interactions leading to both qualitative and quantitative changes in TFII D binding characteristics have been described for several activators (15–20). In contrast, the transcriptional activator Sp1 was found to have no effect on the DNA-binding activity of yeast TFII D (55).

The results of mutational analysis of the SV MLP TATA region (Fig. 3) are consistent with previous reports (45) that basal promoter efficiency is dictated largely by TFII D binding affinity. Interestingly, increased TFII D binding affinity and, therefore, more efficient basal transcription did not abrogate the ability of LSF to activate transcription on the SV MLP. Direct evidence that LSF has no detectable effect on the binding of TFII D was provided by measuring the rates of association and dissociation of TFII D and DNA in the absence and presence of LSF. No apparent effect of bound LSF on either measurement was evident. These results are consistent with our model (Fig. 7) that LSF has no detectable direct effect on the binding of TFII D. Subtle changes in the structure of the TFII D-DNA complex which could be significant in terms of complex function, however, cannot be ruled out completely. These might not be detectable in the gel band mobility shift assay.

**Model for the Mechanism of LSF Transcriptional Activation**—Fig. 7 summarizes our findings on both the SV MLP and the Ad MLP in a flow scheme of the molecular events leading to initiation of transcription. The binding of the TATA-box factor, TFII D, along with TFII A, is relatively slow (9, 55) (Figs. 4B and 7). Depending on the DNA sequence, the association is either stable (TATA consensus mutant SV MLP, Ad MLP) or unstable (wild-type SV MLP). The contribution of TFII A to the formation of a complex of TFII D with the DNA is not clear, but it is likely to stabilize the complex (9, 48). We find that the transcriptional activator LSF has little or no effect on this step in assembly of initiation complexes, because the rate of new complex assembly is slow on a promoter with a weak TFII D binding site, even in the presence of LSF (Fig. 6, panel iii). In addition, prebinding of TFII D has little effect on transcriptional activation by LSF (Fig. 6, panels i versus ii).

Instead, we demonstrate that the subsequent step in initiation complex formation, the binding of TFII B to the TFII A-TFII D-DNA complex (11) (Fig. 7), is also a slow and perhaps rate-limiting step in initiation complex formation. Indeed, preincubation of TFII B greatly enhanced the rate of complete initiation complex formation in the basal assay, indicating that the assembly of TFII B onto the promoter is a prerequisite for high rates of complex assembly. LSF increases the rates of complex formation 30-fold in reactions that have not been preincubated with TFII B. Thus, LSF allows the reaction to rapidly overcome the slow, but prerequisite TFII B binding step. The data are consistent with a model (Fig. 7) whereby LSF activates transcription by facilitating TFII B assembly into the initiation complex. In particular, the model invokes that LSF increases the rate of binding of TFII B to the complex (k2 is greater than k1).

By mass action arguments, driving the TFII B binding reaction forward would also increase the number of TFII A-TFII D-TFII B-DNA complexes (e.g. increase the overall association binding constant for the complex). Thus, it is totally consistent that LSF could both increase the rate of complex formation as evidenced by the kinetic assays (Fig. 6) and also increase the number of complexes formed in the assays that reflect complex stability (Fig. 5). Prebinding of TFII B dramatically decreases activation by LSF (see legend to Fig. 6), but does not totally abolish the LSF effect because TFII B is not totally bound in the preincubation reaction. A molecular interpretation of the kinetic studies suggests that LSF may exert its effect by directly interacting with TFII B and/or with the TFII A-TFII D-TFII B-DNA complex. This could occur either by direct and continuous interaction of LSF with the complex or by induction of a conformational change through direct interaction with one or more of the proteins in the complex. If reversal of a new conformation in the complex were sufficiently slow, then the immediate requirement for LSF would be transient, as has been suggested for ATF (15, 16). However, it is likely that LSF remains closely associated with the template. Binding of LSF to the template is relatively fast and is complete in less than 1 min (Fig. 4B); whereas the half-time for dissociation of the LSF-DNA complex is relatively long, being 8 min.

We have outlined the most logical, simple model (Fig. 7) that is consistent with all of the data presented. More complicated scenarios are possible, but it is striking that invoking an increased k2 versus k1 is sufficient to explain all of our observations. Of course, the interpretation is limited by the fact that the TFII B used in most of the experiments is not homogeneous. However, to date, only one basal promoter activity has been isolated from a TFII B fraction analogous to that used for these studies, although further purification steps were enlisted (27, 48). In addition, we have successfully sub-

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8 D. Hawley, personal communication.
4 R. Hung and R. Sundseth, unpublished observations.
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stituted a highly purified fraction of the rat analogue of TFIIIB, α, for both basal and LSF-activated transcription (data not shown), which strongly argues that there is only one relevant factor in our TFIIIB preparations. One prediction of the model is that the rate-limiting binding of TFIIIB should be overcome by addition of saturating levels of TFIIIB after the preincubation with TFIIA and TFIIID alone, since the rate of a binding reaction is directly proportional to the concentration of substrate. Addition of saturating levels of rat α eliminated the lag in initiation on the Ad MLP due to the slow binding of TFIIIB (α) (data not shown), demonstrating that the slow step detected by the kinetic assays is in fact the binding event and not a subsequent isomerization or conformational change, the rate of which would be independent of concentration. A kinetic analysis of initiation events on the Ad MLP using an approach analogous in design to those presented here also suggests that the binding of TFIIIB is relatively slow (28). TFIIB and RNA pol II have been reported to interact weakly in solution (27); thus, the positioning of TFIIIB in the initiation complex might be a key prior step in the recruitment of RNA pol II onto the promoter (11). Given that TFIIIB does not appear to remain at the promoter after initiation (52), LSF may play an important role in stimulating reinitiation by recruiting TFIIB.

Attempts to directly measure the increased rate of binding of TFIIIB by the presence of LSFW were thwarted, because direct measurement of formation of the TFIIID-TFIIA-TFIIIB-DNA complex by gel band mobility shift analysis requires the use of high levels of TFIIIB that are effectively saturating in the binding reaction. Therefore, as would be expected, the measured rate of TFIIIB assembly onto the TFIIID-TFIIA complex was very fast (less than 1 min, data not shown and data cited in Ref. 48). This is not inconsistent with our transcription assay results, however, demonstrating slow assembly of TFIIIB, as these experiments were done under conditions of limiting TFIIIB where the rate of binding of TFIIIB is expected to be slower. In vivo DNase I footprint analysis of some active promoters demonstrate that only TATA-box occupancy, presumably by TFIIID, is detectable within the basal promoter (56–58), suggesting that in the steady state, the remaining initiation factors and RNA pol II are not bound. The reason for minimal promoter occupancy could be manifold, and is likely to be the result of limiting amounts of factors in the cell. Thus, the ability of an activator such as LSF to nucleate complete initiation complex assembly would represent a sensitive mechanism for attracting these limiting factors ultimately leading to activation of the promoter.

Recently, a new class of proteins termed adaptors or coactivators has been theorized to physically link upstream activators to the RNA pol II general transcription factor machinery and to mediate their activation function (59–65). The possibility that activation of transcription by LSFW may require an adaptor is not inconsistent with the results presented here. An "LSF adaptor" could simply mediate the ability of LSF to increase the rate of TFIIIB binding and serve as a stabilizing force for the TFIIA-TFIIID-TFIIIB-DNA complex.

The kinetics of initiation complex assembly on the basal Ad MLP and SV MLP are strikingly similar, suggesting that these basal promoters function by similar mechanisms. This is not surprising since the two promoters require the same set of general transcription factors, and, though different in sequence, appear to contain similarly functioning basal elements. We conclude that the mechanism for basal promoter function may be similar for many RNA pol II promoters, with the binding of TFIIIB  playing a major role in limiting the rate of initiation. The control point of initiation of basal transcription by transcriptional activating factors could be at any slow step along the pathway of assembly of the rapid-start complex. The SV MLP transcriptional activator, LSF, appears to activate this process at one of its slowest steps, thereby providing the means to rapidly and efficiently induce initiation of transcription from an otherwise inefficient promoter.

Acknowledgments—We are grateful to W. McClure, C. R. Wobbe, J. Licht, and N. DeLuca for critical reading of the manuscript and C. R. Wobbe and P. A. Sharp for many enlightening discussions. We thank C. R. Wobbe and M. K. Shirra for help in preparing protein fractions, P. Casaz, S. Batson, and S. Rimska for various plasmid constructs, R. Hung for help with DNA-binding assays, J. Workman for providing a fraction of TFIIIB, and J. and R. Conaway for providing the rat α factor.

REFERENCES

1. Matsu, T., Segall, J., Weil, P. A., and Roeder, R. G. (1980) J. Biol. Chem. 255, 11992–11996
2. Samuels, M., Fire, A., and Sharp, P. A. (1982) J. Biol. Chem. 257, 14419–14427
3. Dignam, J. D., Martin, P. L., Shasby, B. S., and Roeder, R. G. (1983) Methods Enzymol. 101, 582–598
4. Flores, O., Maldonado, E., Burton, Z., Greenblatt, J., and Reinberg, D. (1988) J. Biol. Chem. 263, 10812–10816
5. Zheng, X.-M., Moncollin, V., Egly, J.-M., and Chambon, P. (1987) Cell 50, 361–368
6. Sumimoto, H., Ohkuma, Y., Yamamoto, T., Horikoshi, M., and Roeder, R. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9158–9162
7. Sawadogo, M., and Roeder, R. G. (1985) Cell 43, 165–175
8. Nakajima, N., Horikoshi, M., and Roeder, R. G. (1988) Mol. Cell. Biol. 8, 4029–4040
9. Fire, A., Samuels, M., and Sharp, P. A. (1984) J. Biol. Chem. 259, 2509–2516
10. Van Dyke, M. W., Sawadogo, M., and Roeder, R. G. (1989) Mol. Cell. Biol. 9, 342–344
11. Burawaski, S., Hahn, S., Guarenie, L., and Sharp, P. A. (1989) Cell 56, 549–561
12. Sawadogo, M., and Sentencas, A. (1990) Annu. Rev. Biochem. 59, 711–754
13. Smale, S. T., and Baltimore, D. (1989) Cell 57, 103–113
14. Sawadogo, M. (1988) J. Biol. Chem. 263, 11994–12001
15. Hai, T., Horikoshi, M., Roeder, R. G., and Green, M. R. (1988) Cell 54, 1043–1051
16. Horikoshi, M., Hai, T., Lia, Y.-S., Green, M. R., and Roeder, R. G. (1988) Cell 54, 1033–1042
17. Horikoshi, M., Carey, M. F., Kakidani, H., and Roeder, R. G. (1988) Cell 54, 665–669
18. Stringer, K. F., Ingler, C. J., and Greenblatt, J. (1990) Nature 345, 783–786
19. Abmayr, S. M., Workman, J. L., and Roeder, R. G. (1988) Genes & Dev. 2, 542–553
20. Leong, K., Brunet, L., and Berk, A. J. (1988) Mol. Cell. Biol. 8, 1765–1774
21. Lin, Y.-S., and Green, M. R. (1991) Cell 64, 971–981
22. Ayer, D. E., and Dynan, W. S. (1988) Mol. Cell. Biol. 8, 2021–2033
23. Ayer, D. E., and Dynan, W. S. (1990) Mol. Cell. Biol. 10, 3655–3665
24. Huang, H.-C., Sundseth, R., and Hansen, U. (1990) Genes & Dev. 4, 267–286
25. Brady, J., Radonovich, M., Vodka, M., Natarajan, V., Thoren, M., Das, G., Janik, J., and Salzman, N. P. (1985) Cell 31, 625–633
26. Shapiro, D. J., Sharp, P. A., Wahli, W. W., and Keller, M. J. (1988) DNA (NY) 7, 47–55
27. Reinberg, D., and Roeder, R. G. (1987) J. Biol. Chem. 262, 3310–3321
28. Reinberg, D., Horikoshi, M., and Roeder, R. G. (1987) J. Biol. Chem. 262, 3322–3330
29. Kadonaga, J. T., and Tjian, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5899–5903
Mechanism of LSF Transcriptional Activation

30. Lee, W., Haslinger, A., Karin, M., and Tjian, R. (1987) *Nature* **325**, 368–372
31. Means, A. L., and Farnham, P. J. (1990) *Mol. Cell. Biol.* **10**, 653–661
32. Hodo, H. G., III, and Blatti, S. P. (1977) *Biochemistry* **16**, 2334–2343
33. Conaway, J. W., Bond, M. W., and Conaway, R. C. (1987) *J. Biol. Chem.* **262**, 8293–8297
34. Galas, D. J., and Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3170
35. Fried, M., and Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6525
36. Fromm, M. and Berg, P. (1982) *J. Mol. Biol.* **16**, 2334–2358
37. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
38. Hirt, B. (1975) *J. Mol. Biol.* **92**, 57–72
39. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
40. Hansen, U., and Sharp, P. A. (1983) *EMBO J.* **2**, 2293–2303
41. Laskey, R. A., and Mills, A. D. (1977) *FEBS Lett.* **82**, 314–316
42. Mullis, K. B., and Faloona, F. A. (1987) *Methods Enzymol.* **155**, 335–350
43. Dynan, W. S., and Tjian, R. (1983) *Cell* **32**, 669–680
44. Kessler, M., and Aloni, Y. (1984) *J. Virol.* **52**, 277–285
45. Huang, D.-H., Horikoshi, M., and Roeder, R. G. (1988) *J. Biol. Chem.* **263**, 12596–12601
46. Wobbe, C. R., and Struhl, K. (1990) *Mol. Cell. Biol.* **10**, 3859–3867
47. Hoffmann, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M., and Roeder, R. G. (1990) *Nature* **346**, 387–390
48. Maldonado, E., Ha, I., Cortes, P., Weis, L., and Reinberg, D. (1990) *Mol. Cell. Biol.* **10**, 6335–6347
49. McClure, W. R. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 5634–5638
50. Hawley, D. K., and Roeder, R. G. (1985) *J. Biol. Chem.* **260**, 8163–8172
51. Hawley, D. K., and Roeder, R. G. (1987) *J. Biol. Chem.* **262**, 3452–3461
52. Van Dyke, M. W., Roeder, R. G., and Sawadogo, M. (1988) *Science* **241**, 1325–1328
53. Smale, S. T., Schmidt, M., Berk, A. J., and Baltimore, D. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4509–4513
54. Conaway, J. W., Travis, E., and Conaway, R. C. (1990) *J. Biol. Chem.* **265**, 7564–7569
55. Schmidt, M. C., Zhou, Q., and Berk, A. J. (1989) *Mol. Cell. Biol.* **9**, 3299–3307
56. Becker, P. B., Ruppert, S., and Schutz, G. (1987) *Cell* **51**, 435–443
57. Phillipsen, J. N. J., Hennis, B. C., and AB, G. (1988) *Nucleic Acids Res.* **16**, 9663–9676
58. Abravaya, K., Phillips, B., and Morimoto, R. I. (1991) *Mol. Cell. Biol.* **11**, 586–592
59. Pugh, B. F., and Tjian, R. (1990) *Cell* **61**, 1187–1197
60. Berger, S. L., Cress, W. D., Cress, A., Triezenberg, S. J., and Guarente, L. (1990) *Cell* **61**, 1199–1208
61. Kelleher, R. J., III, Planagan, P. M., and Kornberg, R. D. (1990) *Cell* **61**, 1209–1215
62. Martin, K. J., Lillie, J. W., and Green, M. R. (1990) *Nature* **346**, 147–152
63. Tasset, D., Tora, L., Fromental, C., Scheer, E., and Chambon, P. (1990) *Cell* **62**, 1177–1187
64. Ptashne, M., and Gann, A. A. F. (1990) *Nature* **346**, 329–331
65. Planagan, P. M., Kelleher, R. J., III, Sayre, M. H., Tschochner, H., and Kornberg, R. D. (1991) *Nature* **350**, 436–438