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Accurate and efficient RNA polymerase II transcription with a soluble nuclear fraction derived from *Drosophila* embryos

(*in vitro* transcription/transcription initiation/U1 small nuclear RNA/Krüppel/GAL4–herpes virus protein 16 fusion protein)

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ABSTRACT **We describe the preparation and biochemical properties of a soluble nuclear fraction derived from *Drosophila* embryos. This extract, which can be easily prepared in 2.5 hr, is capable of accurate and efficient RNA polymerase II transcription of a variety of diverse genes from both *Drosophila* and mammals. With the relatively strong promoter of the *Drosophila* Krüppel gene, it is possible to achieve 20% template usage in a single round of transcription, which is considerably higher than the template usage of ≈3% seen with standard nuclear extracts. Further, although U small nuclear RNA genes are refractory to transcription with HeLa transcription extracts, the soluble nuclear fraction transcribes a U1 small nuclear RNA gene from *Drosophila*. Moreover, transcriptional activation by sequence-specific activators can be attained *in vitro* with the soluble nuclear fraction. The overall transcriptional efficiency appears limited to 0.45 transcript per template of DNA per 30 min, but the mechanism of limitation is not known. The soluble nuclear fraction, which was developed to recreate the environment within the nucleus, should be useful when high efficiencies of RNA polymerase II transcription are desired.**

The growth, development, and sustenance of an organism depend upon the regulation of tens of thousands of genes. An important step at which protein-coding genes are turned on and off in eukaryotes is initiation of transcription by RNA polymerase II. To understand the underlying mechanisms by which synthesis of mRNA is controlled, it is necessary to carry out the transcription reaction *in vitro* and then to unravel the identity and function of the participating factors. To this end, *in vitro* transcription systems have been developed from a variety of organisms, and factors involved in both basal and regulated transcription by RNA polymerase II have been characterized. The basic transcription reaction is carried out by RNA polymerase II and several auxiliary factors. These auxiliary or general factors are required for promoter recognition, initiation, and elongation (for recent review, see ref. 1). Regulation of transcription involves, at least in part, the action of sequence-specific DNA-binding factors that interact with promoter and enhancer elements (for recent review, see ref. 2).

A major limitation in analyzing RNA polymerase II transcription has been that *in vitro* transcription systems do not faithfully reproduce transcription seen *in vivo*. For instance, long-range activation *in vitro* occurs with a potent GAL4–herpes virus protein 16 fusion protein (3), but normal enhancer function with factor-binding sites located >1 kilobase (kb) from the RNA start sites has not yet been observed. Also, many genes without A+T-rich segments (TATA boxes) upstream of the RNA start sites cannot be transcribed *in vitro* with the commonly used transcription systems from mammalian cells. Finally, transcription *in vitro* is inefficient: typically, <3% of the DNA templates are used in a single round of transcription. Thus, to better understand transcription by RNA polymerase II, it is important to address and to rectify the shortcomings of existing *in vitro* transcription systems.

We have been developing an *in vitro* system that more faithfully reconstitutes the *in vivo* transcription activities of RNA polymerase II. In these studies, we use *Drosophila* embryos as a source of RNA polymerase II activity. Transcription factors from embryos are highly active (4–6), and embryos can be obtained in kilogram quantities at a relatively low cost. In addition, the general RNA polymerase II transcription factors are functionally conserved from *Drosophila* to humans (7). In this work, we have improved the efficiency of RNA polymerase II transcription *in vitro*. A critical technical advancement was the preparation of a soluble fraction from *Drosophila* embryo nuclei, which we refer to as the soluble nuclear fraction (SNF). In this communication, we examine the strengths and limitations of transcription *in vitro* with the SNF.

MATERIALS AND METHODS

Plasmids and Proteins. Transcription and primer-extension analysis of the *Drosophila* alcohol dehydrogenase gene, the *Drosophila* jockey mobile element, and the adenovirus *E4* gene were carried out with plasmids and oligonucleotides described by Wampler *et al.* (7). Plasmid pKr-B5.5 contains a 5.5-kb *Bam*HI fragment of the *Drosophila* Krüppel gene (*Kr*), which encompasses roughly −4.5 to +1 kb relative to the RNA start sites, inserted into pUC118. The *Drosophila* gene *Kr* was provided by Michael Hoch and Herbert Jäckle (University of Munich) as the λER3 clone (8). Primer-extension analysis of *Kr* transcripts was done as described by Kadonaga (6). Plasmid pUC-U1a contains the promoter region of the *Drosophila* U1-95.1 gene (9) from −388 to +30 relative to the RNA start site inserted into pUC118. The *Drosophila* U1-95.1 gene was the gift of Patrick Lo and Steve Mount (Columbia University). The GAL4 derivatives were expressed in *Escherichia coli* and purified to ≈80% homogeneity according to the procedure of Chasman *et al.* (10). Transcription activation by the GAL4 derivatives was performed with plasmid pG_5E4, which contains five GAL4-binding sites upstream of the TATA box of the adenovirus E4 promoter (11). *In vitro* transcription and primer-extension analysis with pG_5E4 template DNA were done as described elsewhere (12). Transcription factor Sp1 was purified from HeLa cells (13). Transcription activation by Sp1 was done with plasmid pSV-Kr, which contains five Sp1-binding sites [from the three 21-base-pair (bp) repeats of simian virus 40] upstream of the TATA box of the *Kr* minimal promoter (12). *In vitro* transcription and primer-extension analysis with

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Abbreviations: SNF, soluble nuclear fraction; *Kr*, Krüppel gene; snRNA, small nuclear RNA.

pSV-Kr were done as described for the *Kr* minimal promoter (pKr-31/+13) in Kerrigan *et al.* (12). Transcriptional activation by human Fos and Jun proteins purified from *E. coli* (gift of Cory Abate and Tom Curran, Roche Institute of Molecular Biology, Nutley, NJ; ref. 14) was performed with plasmid pAP-1 CAT, which contains four AP-1-binding sites upstream of the basal human metallothionein IIA promoter. *In vitro* transcription and primer-extension analysis with pAP-1 CAT were performed as described by Perkins *et al.* (15).

Buffers and Solutions. All buffers and solutions were prepared from glass-distilled water. Embryo wash solution is 0.7% (wt/vol) NaCl/0.04% (vol/vol) Triton X-100. Buffer I is 15 mM Hepes (K^+), pH 7.6/10 mM KCl/5 mM $MgCl_2$/0.1 mM EDTA/0.5 mM EGTA/350 mM sucrose/1 mM dithiothreitol/1 mM sodium bisulfite/1 mM benzamidine/0.2 mM phenylmethylsulfonyl fluoride. Buffer AB is 15 mM Hepes (K^+), pH 7.6/110 mM KCl/5 mM $MgCl_2$/0.1 mM EDTA/2 mM dithiothreitol/1 mM sodium bisulfite/1 mM benzamidine/0.2 mM phenylmethylsulfonyl fluoride. HEMG20 + 0.4 M potassium glutamate is 25 mM Hepes (K^+), pH 7.6/0.4 M potassium glutamate/12.5 mM $MgCl_2$/0.1 mM EDTA/20% (vol/vol) glycerol/1.5 mM dithiothreitol.

Preparation of the SNF. A typical preparation of the SNF was carried out with 50–150 g of embryos. Canton S wild-type flies were grown at 25°C at 70–80% humidity in population cages, and embryos were deposited on molasses/agar trays covered with yeast and collected between 0 and 12 hr after fertilization. These trays were stored for up to 3 days at 4°C (see ref. 16) after embryo collection. Embryos were harvested in nylon mesh (Tetko, Monterey Park, CA; 3-70/43) with chilled (10–15°C) water and then dechorionated by immersion for 90 sec in dilute bleach [final concentration of sodium hypochlorite is 2.63% (wt/vol)] at room temperature. The embryos were quickly rinsed with the embryo wash buffer (1 liter) and then with chilled (10–15°C) water. The embryos were next dried into a moist cake by blotting with paper towels, weighed, and placed on ice.

Nuclei were prepared similarly to that described by Soeller *et al.* (5) and Wampler *et al.* (7). All operations were performed at 4°C. The embryos were suspended in buffer I (3 ml of buffer per g of embryos) and homogenized by a single passage through a Yamato LH-21 homogenizer at 1000 rpm. The homogenate was filtered through 1 layer of Miracloth (Calbiochem), and the debris retained by the Miracloth was rinsed with additional buffer I (2 ml per g of embryos). The nuclei were pelleted in a Sorvall GSA rotor at 8000 rpm (10,400 × *g*) for 15 min. The supernatant was carefully decanted from the loose pellet of nuclei, and lipid deposits on the sides of the centrifuge bottles were removed with paper tissue (Kimwipes; Kimberly-Clark). The nuclei were resuspended in buffer I (3 ml per g of embryos). The hard yellow yolk pellet was avoided when possible. A 40-ml Dounce homogenizer (Wheaton Scientific) with a B pestle was used to disperse the nuclei. The nuclei were then repelleted in a Sorvall GSA rotor at 8000 rpm for 15 min. The nuclei were then suspended in buffer AB (1 ml per g of embryos) by using a 40-ml Dounce homogenizer with a B pestle. The nuclei were repelleted in a GSA rotor at 8000 rpm for 10 min. The supernatant was discarded, and the mass of the nuclei was measured. It is important to note that transcription factors slowly diffuse out of the nuclei, and thus, it is necessary to wash and to pellet the nuclei as quickly as possible.

The SNF was then prepared from the washed nuclei as follows. To each gram of nuclei, 0.5 ml of HEMG + 0.4 M potassium glutamate was added. The nuclei were suspended by swirling and shaking (the Dounce homogenizer should not be used). The suspension was incubated on ice for 15 min. The mixture was then centrifuged in a Beckman SW28 rotor at 24,000 rpm (100,000 × g_{max}) for 1 hr (in our experience, centrifugation in a fixed-angle rotor did not yield an active transcription extract). After centrifugation, the upper lipid layer was discarded, and the clear supernatant, which is the SNF, was removed with a pipet, frozen in liquid nitrogen, and stored at −100°C. (The SNF can be used directly in transcription reactions and does not need to be dialyzed.) The grey liquid layer below the SNF was avoided. We typically obtain 15–20 ml of SNF per 100 g of embryos. The protein concentration of the SNF was 30 mg/ml, as determined by the method of Bradford (17) with bovine γ-globulin as a reference.

***In Vitro* Transcription Analysis.** *In vitro* transcription reactions and primer-extension analysis of the transcripts were carried out essentially as described by Kadonaga (6). All reactions were performed two to four times to ensure data reproducibility. Transcription reactions were done with either 5 or 20 μl of the SNF in a 26-μl final volume. In the reactions with 5 μl of SNF, the following were present: a solution 5 mM in each of four ribonucleoside triphosphates adjusted to pH 7.0 (3 μl); supercoiled template DNA at 50 ng/μl (2 μl); 0.5 μl of Inhibit-ACE (0.5 unit; 5 Prime → 3 Prime, Inc.); 0.5 μl of RNase Block II (0.25 unit; Stratagene); and 12.5 mM Hepes (K^+), pH 7.6, 0.1 M potassium glutamate, 6.25 mM $MgCl_2$, 0.05 mM EDTA, 5% (vol/vol) glycerol, and 0.5 mM dithiothreitol (15 μl). In reactions with 20 μl SNF, the following were present: a solution 5 mM in each of four ribonucleoside triphosphates adjusted to pH 7.0 (3 μl); supercoiled template DNA at 50 ng/μl (2 μl); 0.5 μl of Inhibit-ACE (0.5 unit; 5 Prime → 3 Prime, Inc.); and 0.5 μl of RNase Block II (0.25 unit; Stratagene).

The quantities of RNA synthesized were determined as follows. The sections of the gels corresponding to the reverse transcription products and ^{32}P-labeled DNA fragments, which were standards, were excised and soaked in a mixture of 3% (vol/vol) Protosol (New England Nuclear) in Aquasol-2 (New England Nuclear). The amount of radioactivity in each sample was then measured by scintillation counting. The ^{32}P-labeled standards were the synthetic oligonucleotides that were used in the primer-extension analyses. The concentration of the oligonucleotides was estimated by determination of the A_{260} values of the samples, assuming that oligonucleotide at 1 mg/ml corresponds to 25 *A* units at 260 nm.

RESULTS AND DISCUSSION

The SNF. As a first step toward increasing the efficiency of transcription by RNA polymerase II *in vitro*, we sought to prepare an extract that recreates the soluble environment within the nucleus. In general, procedures for preparation of RNA polymerase II transcription factors (see, for example, refs. 5 and 18–24), which typically involve salt extraction of transcription factors from nuclei or cell lysates, significantly dilute the factors relative to their concentration in the nucleus. To minimize such dilution, we have developed a procedure that releases soluble factors from nuclei by centrifuging a nuclear pellet at high speed. We refer to the supernatant of the centrifugation as the SNF.

We examined a variety of conditions for SNF preparation and tested the fraction activity by carrying out transcription reactions with the promoter of the *Drosophila Kr* gene. The *Kr* promoter is a relatively strong promoter *in vitro* and contains several RNA start sites clustered over a 10-bp region (6, 12). After optimizing the conditions for washing and centrifugation of the nuclei, we determined the variation of transcription activity with the concentration of *Kr* template DNA (Fig. 1). As SNF concentration was increased from 30 to 600 μg of total protein per 25-μl reaction, the total amount of transcription progressively increased; Fig. 1 shows the results of reactions done with 5 μl (150 μg of total protein) and 20 μl (600 μg of protein). Synthesis of RNA with the SNF was

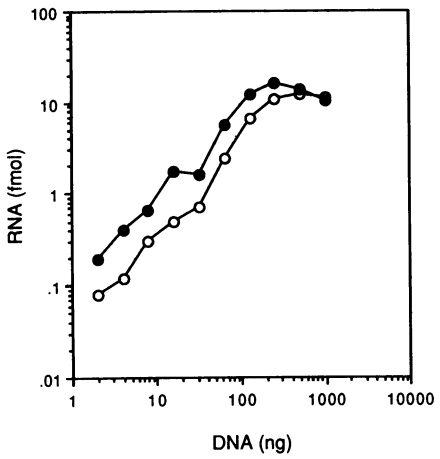

FIG. 1. Effect of template DNA concentration upon transcription by the SNF. The SNF (5 or 20 μl) was preincubated with indicated amounts of pKr-B5.5 template DNA for 15 min to assemble initiation complexes, and the transcription reactions were initiated by adding ribonucleoside triphosphates and were terminated after 30 min. Synthesis and analysis of the transcripts were done as described. Amount of template DNA (in ng) in the reaction is plotted against transcripts synthesized (in fmol). ○, 5 μl of SNF; ●, 20 μl of SNF.

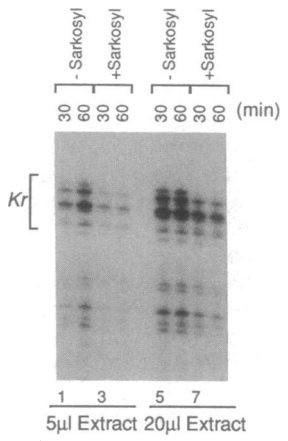

FIG. 2. Efficiency of transcription with the SNF. Transcription initiation complexes were assembled on pKr-B5.5 template DNA (100 ng) by incubation at 21°C for 30 min. Either 5 or 20 μl of SNF was used in the reactions, as indicated. The reactions were initiated by adding ribonucleoside triphosphates and proceeded for either 30 or 60 min. In the reactions containing Sarkosyl, the detergent was added to 0.25% (wt/vol) final concentration 10 sec after adding the ribonucleoside triphosphates. Reverse transcription products of *Kr* RNA are shown.

also sensitive to α-amanitin at 4 μg/ml, which demonstrated that transcription was carried out by RNA polymerase II (data not shown). The amount of RNA synthesized increased linearly with concentration of the *Kr* DNA from 2 to 256 ng of template DNA in a 25-μl reaction. This linearity between transcription and template concentration indicates that the SNF contains negligible levels of nonspecific DNA-binding factors that inhibit transcription. In contrast, we have found that *Drosophila* nuclear extracts prepared by conventional salt extraction (4, 5) contain significant levels of nonspecific DNA-binding factors that inhibit transcription (ref. 12; data not shown).

Efficiency of Transcription *In Vitro*. The performance of an *in vitro* transcription system is determined by the fraction of templates used in a single round of transcription and by the number of rounds of transcription that are done. Using a standard *Drosophila* nuclear extract (5), we had previously found that transcription was linear through the first 60 min and that 3% of the templates undergo about six rounds of transcription in 1 hr to yield an overall efficiency of 0.2 transcript per template per hr (6). To measure these parameters with the SNF, we used the detergent Sarkosyl, which permits elongation by RNA polymerase II but inhibits assembly of new initiation complexes (6, 25, 26). In these experiments, transcription initiation complexes were assembled on the *Kr* template DNA, ribonucleoside triphosphates were added to initiate transcription, and in some instances, Sarkosyl was subsequently added to 0.25% (wt/vol) to limit transcription to a single round (Fig. 2). We observed 18–22% template use (in a single round of transcription) with a high SNF concentration (Fig. 2, lanes 7 and 8) and ≈6% template use with a lower SNF concentration (Fig. 2, lanes 3 and 4). Thus, the template use is severalfold higher with the high SNF concentration than with the standard nuclear extract. Transcription was linear for 60 min with low SNF (5 μl per 25-μl reaction) and for only 30 min with high SNF (20 μl per 25-μl reaction) (data not shown). Curiously, under many different reaction conditions, a plateau of ≈0.45 transcript per template could not be exceeded (see, for example, Fig. 2, lanes 5 and 6). This limitation could be due to template or factor inactivation during transcription, depletion, or degradation of substrates and cofactors, or perhaps the generation of a transcription inhibitor (for example, end-product inhibition). We have explored many of these possibilities, but we have not yet determined the nature of the transcription inhibition. Nevertheless, transcription with the SNF is highly efficient when compared with other RNA polymerase II systems. With high SNF concentrations, transcription reactions typically yield 0.45 transcript per template per 30 min, which is higher than 0.1 transcript per template per 30 min (6) and 0.005–0.02 transcript per template per 30 min (4) that have been determined previously with other *Drosophila* embryo extracts. Transcription with HeLa factors typically yields <0.05 transcript per template per 30 min (19, 20, 27, 28). A procedure for the preparation of a HeLa transcription system that produces 1.4–4 transcript per template has been described (24), but we have not been able to increase transcription efficiency by using a similar method with *Drosophila* embryos (data not shown).

The SNF Can Transcribe Diverse Genes, Including a U1 Small Nuclear RNA (snRNA) Gene. We investigated transcription of a diverse set of genes to test the generality of transcription by the SNF. As shown in Fig. 3*A*, *Drosophila Kr*, which is involved in the early steps of anterior–posterior segmentation of the embryo (29), the *Drosophila* alcohol dehydrogenase gene (proximal promoter; ref. 30), the adenovirus *E4* gene, and the *Drosophila* jockey mobile element, which contains an unusual internal promoter (31), are accurately transcribed by the SNF. RNA synthesis was inhibited by α-amanitin at 4 μg/ml which indicates that transcription was carried out by RNA polymerase II.

We also examined transcription of a *Drosophila* U1 snRNA gene (9, 32). U1 snRNA is an abundant snRNA involved in the process of pre-mRNA splicing. The U snRNA genes, with the exception of U6, are transcribed by RNA polymerase II (U6 is transcribed by RNA polymerase III). Although transcription of protein-coding genes by the RNA polymerase II apparatus can be done with fractionated and partially purified factors, it has generally been difficult to recreate transcription of the snRNAs *in vitro* (33). At present, *in vitro* synthesis of U snRNAs has been documented with a *Xenopus* extract of germinal vesicles isolated under oil (34), a concentrated whole-cell extract from *Ascaris* embryos (35), and nuclear extracts derived from sea urchins (36, 37). In contrast, the well-characterized RNA polymerase II system from HeLa cells does not transcribe the snRNA genes (33). We have found that the SNF can accurately initiate transcription from

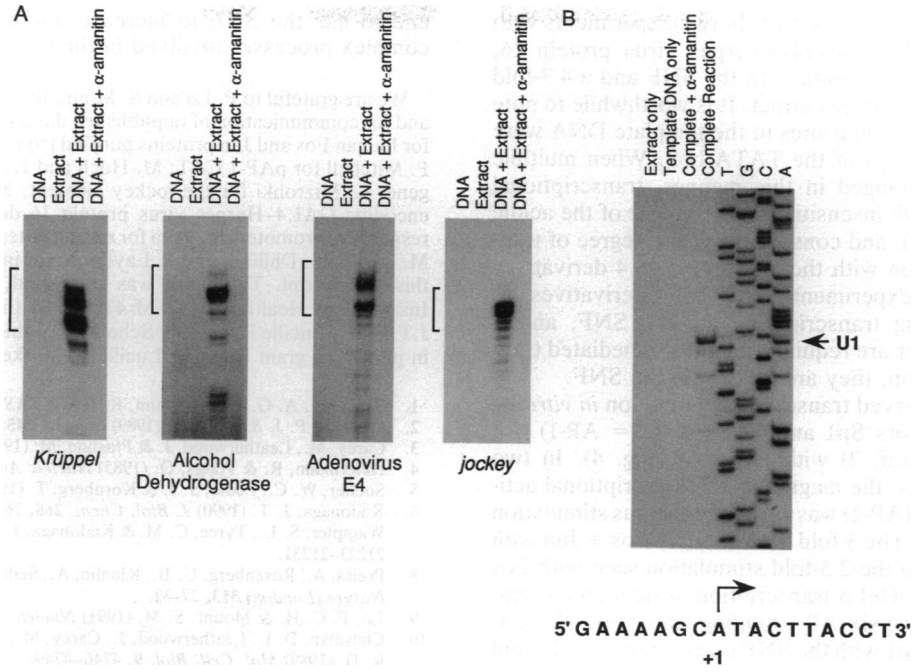

FIG. 3. Transcription of diverse genes with the SNF. Standard transcription reactions were done with the indicated template DNAs (100 ng) and the SNF (5 μl). Where indicated, α-amanitin was added to 4 μg/ml (final concentration) to inhibit transcription by RNA polymerase II. Reverse transcription products of the *in vitro*-synthesized transcripts are shown. (*A*) Transcription of *Drosophila Kr*, the *Drosophila* alcohol dehydrogenase gene (proximal promoter), the adenovirus *E4* gene, and the *Drosophila* jockey mobile element. (*B*) Transcription of the *Drosophila* U1-95.1 gene (U1). The DNA sequence encompassing the RNA start site is shown at bottom.

a *Drosophila* U1 snRNA promoter (Fig. 3*B*). Transcription was inhibited by α-amanitin at 4 μg/ml and was thus done by RNA polymerase II. The efficiency of RNA synthesis was 0.01 transcript per template per 30 min, which is similar to that of weak *Drosophila* promoters. We did not examine capping or 3'-end formation. *In vitro* transcription of the *Drosophila* U1 gene was not, however, specific for the SNF. We also found that a conventional nuclear extract from *Drosophila* embryos (7) could accurately transcribe initiation of the U1 gene (data not shown), but transcription of the U1 gene was 5-fold less efficient with the standard extract (0.002 transcript per template per 30 min) than with the SNF. Because the general RNA polymerase II transcription factors are conserved from yeast to humans, these data suggest there may be a simple explanation (reaction condition, missing factor, etc.) for the lack of U snRNA transcription with the HeLa system. Furthermore, the differences, if any, between transcription of protein coding versus U snRNA genes can now be easily tested.

Transcription Activation with Sequence-Specific DNA-Binding Factors. We then investigated whether or not promoter- and enhancer-binding factors can activate transcription *in vitro* when used in conjunction with the SNF. Transcriptional activation with sequence-specific DNA-binding activators has been suggested to require auxiliary factors, referred to as adapters, mediators, or coactivators, that serve as an intermediary bridge between the DNA-bound factors and the RNA polymerase II complex (38–42). If such auxiliary factors are required for transcription activation, they may not be present in the SNF. To test this possibility, we carried out transcription reactions with derivatives of yeast GAL4 protein, which is a sequence-specific DNA-binding protein with an acidic transcription activation motif (for review, see ref. 43). The following GAL4 derivatives, which can all bind specifically to GAL4 recognition sites, were used: (*i*) GAL4(1–147), which possesses a cryptic activation domain; (*ii*) GAL4(1–147)AH, which contains an amphipathic helix that functions as an activation domain; and (*iii*) GAL4(1–147)–herpes virus protein 16, which possesses a strong activation domain. Using purified GAL4 proteins with a template containing five GAL4-binding sites upstream of the TATA box of the adenovirus *E4* gene (11), we found substantial activation of transcription by the GAL4 derivatives with both the standard nuclear extract and the SNF (Fig. 4). Transcriptional activation was binding site-dependent (data not shown). The degree of transcriptional stimulation attained with the SNF was slightly higher than

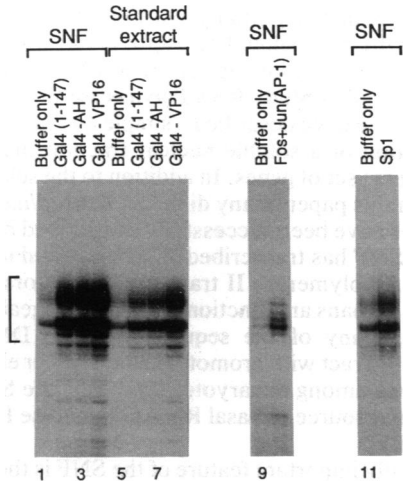

FIG. 4. *In vitro* activation by GAL4 derivatives, Sp1, and Fos + Jun (AP-1) with the SNF. Transcription reactions were done with template DNA (100 ng), standard nuclear extract (5 μl; ref. 5), or SNF (5 μl), and the indicated sequence-specific transcription factors (100 ng each of GAL4 derivatives; 35 ng of Sp1; 80 ng of Jun plus 75 ng of Fos). Template DNAs were preincubated on ice with the sequence-specific factors for 15 min before SNF addition. Reactions were initiated by adding ribonucleoside triphosphates and were allowed to proceed for 30 min at 21°C. Template DNAs used in the transcription reactions are as described. Reverse transcription products of the RNAs are shown. VP16, Herpes virus protein 16.

that seen with the standard extract. In two experiments with the fusion protein GAL4(1–147)–herpes virus protein 16, there was a 7.9-fold activation with the SNF and a 4.3-fold activation with the standard extract. It is worthwhile to note that the five GAL4-binding sites in the template DNA were in the proximity (23 bp) of the TATA box. When multiple binding sites are arranged in this manner, transcriptional activation is relatively insensitive to the nature of the acidic activating region (44), and consequently, the degree of transcriptional stimulation with the different GAL4 derivatives was similar in these experiments. The GAL4 derivatives are capable of activating transcription with the SNF, and if adapters or mediators are required for GAL4-mediated transcriptional stimulation, they are present in the SNF.

We have also observed transcription activation *in vitro* by the mammalian factors Sp1 and Fos + Jun (= AP-1) (for recent review, see ref. 2) with the SNF (Fig. 4). In two separate experiments, the magnitude of transcriptional activation by Fos + Jun (AP-1) was 3.0-fold, whereas stimulation by Sp1 was 2.4-fold. The 3-fold activation by Fos + Jun with the SNF is similar to the 2.5-fold stimulation seen with Fos + Jun when using a HeLa transcription system and a template DNA containing six AP-1-binding sites (14). The 2.4-fold stimulation by Sp1 with the SNF is less than the >10-fold activation typically observed *in vitro* (2). However, when Sp1 activation is measured by using preparations of general transcription factors devoid of nonspecific DNA-binding proteins (such as histone H1) that inhibit transcription, a high level of basal transcription occurs in the absence of Sp1, and the magnitude of Sp1-mediated activation is only ≈2.5- to 5-fold (see, for example, refs. 38, 39, and 42). As a result, the 2.4-fold activation by Sp1 with the SNF, which appears devoid of nonspecific DNA-binding inhibitors of transcription (see previous discussion of the data in Fig. 1), is similar to the 2.5- to 5-fold stimulation observed in experiments with partially purified HeLa transcription systems (38, 39, 42). Furthermore, it is possible to reconstitute >10-fold Sp1-mediated activation by adding purified histone H1 to the SNF to repress basal transcription (G. E. Croston and J.T.K., unpublished data). Hence, the properties of the SNF are similar to that of the partially purified HeLa transcription factors, and the SNF should be useful in the analysis of both basal and regulated transcription by RNA polymerase II.

Use of the SNF to Study RNA Polymerase II Transcription. In summary, we have described a simple and rapid procedure for preparation of a soluble nuclear fraction that can transcribe a diverse set of genes. In addition to the selected genes described in this paper, many different *Drosophila* and mammalian genes have been successfully transcribed by the SNF. In fact, the SNF has transcribed all genes tested to date. The general RNA polymerase II transcription factors from *Drosophila* and humans are functionally interchangeable (7), but, in contrast, many of the sequence-specific DNA-binding factors that interact with promoter and enhancer elements are not conserved among eukaryotes (2). Thus, the SNF should be viewed as a source of basal RNA polymerase II transcriptional activity.

A potentially important feature of the SNF is the efficiency of template use in a single round of transcription. It may now be possible to examine new aspects of transcription initiation and reinitiation with the SNF. For instance, we have found that 20% of the templates are transcribed only twice with the SNF, whereas 3% of the templates can be transcribed 10 times with standard extracts (6). Is the SNF deficient in a factor required for reinitiation of transcription? Alternatively, is it possible to increase the overall transcription efficiency by adjusting for substrate depletion or end-product inhibition? We hope, in the future, to address such questions and to use the SNF to increase our understanding of the complex processes involved in the transcription reaction.

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