Oct-2 facilitates functional preinitiation complex assembly and is continuously required at the promoter for multiple rounds of transcription

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Octamer factor 2 (Oct-2, OTF-2, NF-A2) is an 'upstream' promoter factor that binds to the octamer motif (ATGCAAAT) implicated in control of immunoglobulin gene transcription in B-lymphocytes. We have studied the role of Oct-2 in the process of transcription initiation *in vitro* using both nuclear extracts and purified basal transcription factors. Oct-2 specifically stimulates transcription from octamer-containing promoters in both systems. Thus, Oct-2 is a 'true activator', rather than merely an 'anti-repressor' counteracting the effect of histones. In order-of-addition experiments, Oct-2 is required early, together with TFIID, to allow formation of a preinitiation complex. Oct-2 cannot functionally interact with cloned TATA binding protein (TBP) but rather requires 'coactivators' found in the TFIID fraction. In single-round transcription experiments, early competition for Oct-2 by an octamer oligonucleotide is deleterious, but no effect is seen after assembly of a complete preinitiation complex. However, for multiple rounds of transcription, Oct-2 is continuously required at the promoter; this result argues against a 'hit-and-run' mechanism whereby the activator becomes dispensable after organizing a TFIID-promoter complex. In agreement with our previous studies *in vivo*, the N-terminal glutamine-rich activation domain of Oct-2 is required for full activity *in vitro*, indicating that this domain directly interacts with basal transcription factors. Key words: glutamine domains/*in vitro* transcription/Oct-2/transcriptional activation

Introduction

An important regulatory sequence in immunoglobulin gene promoters and the heavy chain enhancer is the octamer motif ATGCAAAT (reviewed in Kemler and Schaffner, 1990); this motif is also present in non-tissue-specific promoters including the histone H2B and certain snRNA promoters (Carbon *et al.*, 1987; LaBella *et al.*, 1988). The ubiquitous Oct-1 (Sturm *et al.*, 1988) and the lymphocyte-specific Oct-2 factors (Clerc *et al.*, 1988; Ko *et al.*, 1988; Müller *et al.*, 1988; Scheidereit *et al.*, 1988) bind to and activate promoters via the octamer sequence. Additional cell type-specific octamer binding factors have been described (Schreiber

et al., 1990; Schöler, 1991). Different octamer factors can bind the same sequence yet carry out presumably distinct functions. Mutational analysis has indicated which segments of octamer factors are important for activation (Gerster *et al.*, 1990; Müller-Immerglück *et al.*, 1990; Tanaka and Herr, 1990; Annweiler *et al.*, 1992; Tanaka *et al.*, 1992), but targets of octamer factors have not been identified.

Activation of transcription is still poorly understood, although recent progress suggests that there are multiple mechanisms. According to the terminology of Croston *et al.* (1991), activation can be classified as 'anti-repression', i.e. counteracting the negative effects of chromatin or other components, or 'true activation', i.e. direct interaction with elements of the basal transcription apparatus. Several activators have been found to interact with TFIID, the multifactor complex which binds the TATA box. ATF and chimeric GAL4 derivatives have been found to alter TFIID footprinting patterns, while the pseudorabies immediate early protein and the EBV Zta protein have been found to facilitate TFIID-DNA interaction (Abmayr *et al.*, 1988; Horikoshi *et al.*, 1988a,b; Stringer *et al.*, 1990; Lieberman and Berk, 1991). TFIIB also appears to be a target of activators. A GAL4-VP16 chimeric activator bound TFIIB directly and promoted stable association of TFIIB with the preinitiation complex (Lin and Green, 1991; Lin *et al.*, 1991), while LSF, a factor which stimulates transcription from the SV40 major late promoter, increased the rate of TFIIB binding to the preinitiation complex (Sundseth and Hansen, 1992).

The role of an activator after formation of the preinitiation complex remains to be elucidated. Experiments with the ATF activator suggested that once a transcription complex has been established, ATF can be removed without impairing transcription (Hai *et al.*, 1988), implying that once activated, a target promoter would remain 'on'. In contrast, experiments with GAL4-VP16 suggested that antibody inhibition of the VP16 moiety shuts down transcription (White *et al.*, 1992), although in this case, the promoter-bound antibody molecules may themselves be inhibitory.

In order to make functional comparisons between Oct-2 and other factors, we have initiated mechanistic studies to understand how Oct-2 activates transcription *in vitro* and to identify the targets of this factor. We found that Oct-2A (referred to hereafter as Oct-2) acts together with TFIID to form preinitiation complexes, and that Oct-2 must remain at the promoter for multiple rounds of initiation. Glutamine-rich activation domains similar to those of Oct-2 are found in other transcription factors (Mitchell and Tjian, 1989), thus these factors may all operate by a common mechanism.

Results

Activation of *in vitro* transcription by Oct-2

To study activation by the Oct-2 factor, test templates were transcribed *in vitro* using nuclear extracts derived from BJA-B (B-lymphocyte) or HeLa cells. A κ light-chain promoter

Fig. 1. (A) Oct-2 activation of an immunoglobulin κ light-chain promoter. Plasmid p $\kappa\beta$ +21 (200 ng) bearing a -400 to +21 fragment of the MOPC-41 κ light-chain promoter (Picard and Schaffner, 1985) was transcribed with HeLa nuclear extract in the presence of 0, 1.7, 3.3 or 6.7 pmol Oct-2 (lanes 1–4) and transcripts were mapped by RNase protection. (B) Oct-2 activation of a composite octamer promoter in BJA-B and HeLa nuclear extracts. Plasmid p70Zhep-oct⁺ (400 ng) containing a single octamer binding site upstream of the rabbit β globin TATA box (Kemler *et al.*, 1989) and the OVEC-ref plasmid (400 ng) driven by an SV40 enhancer sequence (Westin *et al.*, 1987) were transcribed in HeLa or BJA-B nuclear extracts in the presence of 0, 0.7, 1.6 or 6.7 (lanes 1–4) pmol Oct-2 and transcripts were mapped by S1 nuclease analysis.

(Figure 1A) and a composite promoter consisting of a single octamer site adjacent to the rabbit β globin TATA box (Figure 1B) were activated up to 10-fold by addition of recombinant Oct-2 produced in *Escherichia coli*. Oct-2 stimulated transcription to similar maximal levels in both B-lymphocyte and non-lymphocyte systems (Figure 1B), indicating that no B cell-specific factors are required for Oct-2 activity, in accord with other *in vitro* studies (LeBowitz *et al.*, 1988; Johnson *et al.*, 1990; Pierani *et al.*, 1990) and with *in vivo* results obtained from transfections (Kemler *et al.*, 1991; Yang *et al.*, 1991). Basal transcription from octamer promoters was higher with BJA-B extracts than with HeLa extracts (Figure 1B), most likely because of endogenous Oct-2 in the BJA-B extract. A reference promoter lacking octamer binding sites was not activated by Oct-2 (Figure 1B), indicating that the activation was specific.

It has been proposed that B-cell-specific factors further enhance octamer activation on some promoters; octamer factors from B-cell nuclear extracts drive transcription *in vitro* from an immunoglobulin heavy chain promoter and an MHC II DRA promoter more efficiently than do octamer factors from HeLa extracts (Pierani *et al.*, 1990; Zeleznik-Le *et al.*, 1992). This and other studies show, however, that Oct-2 has an intrinsic ability to stimulate transcription. In fact, the Oct-2 glutamine-rich activation domain can by itself stimulate transcription when fused to a heterologous DNA binding domain (Seipel *et al.*, 1992).

Oct-2 facilitates formation of preinitiation complexes

Since transcription can be influenced at numerous points both prior to and following initiation (Kerppola and Kane, 1991; Roeder, 1991), we investigated at which stage Oct-2 acts using single-round transcriptions. This technique uses a detergent block; low levels of Sarkosyl do not disrupt fully formed preinitiation complexes but do prevent reinitiation by RNA polymerase. The amount of RNA produced is proportional to the number of preinitiation complexes formed prior to the block (Hawley and Roeder, 1987). If Oct-2 acts

Fig. 2. (A) Oct-2 facilitates formation of preinitiation complexes in single-round transcription assays. Nuclear extract, octamer promoter plasmid p70Zhep-oct⁺ (400 ng) and OVEC-ref (400 ng) were preincubated in transcription buffer at 30°C for 20 min before addition of NTPs and Sarkosyl detergent. Transcriptions were stopped at either 10, 20 or 40 min and RNA analyzed by S1 nuclease analysis. Oct-2 (6.7 pmol) was added at 0 min ('early') (lanes 4–6) or at 20 min ('late') (lanes 7–9). (B) Kinetic scheme for single-round transcription assays.

prior to initiation of transcription, then the factor should increase the number of transcription complexes formed in the absence of nucleoside triphosphates (NTPs). HeLa nuclear extract was incubated for 20 min with a test octamer promoter in the presence or absence of Oct-2, then NTPs and detergent were added, and the transcription reactions were stopped after 10, 20 or 40 min. As shown in Figure 2, the presence of Oct-2 stimulated preinitiation complex formation in the absence of NTPs to an extent comparable with that seen in multiple-round transcriptions (e.g. Figure 1B, ~10-fold). Addition of Oct-2 after the detergent block had no effect. Elongation reactions of 10, 20 and 40 min yielded identical amounts of the specifically initiated transcript, indicating that no further initiation was occurring and that the RNA was stable. Readthrough transcripts accumulated slowly as polymerase molecules that had initiated elsewhere on the plasmid transcribed through the mapped region. A slight increase in the reference signal was noted (lanes 4–6), which seems to be due to breakdown from full-length signal at an S1 sensitive site (data not shown).

Analysis of promoter complex formation has shown that after binding of basal transcription factors, ATP hydrolysis precedes or occurs simultaneously with initiation by RNA polymerase II (Bunick *et al.*, 1982; Sawadogo and Roeder, 1984). Thus, Oct-2 facilitates the formation of transcription complexes prior to this ATP-requiring step.

Oct-2 functions as a 'true activator'

Stimulation of preinitiation complex formation might be the result of direct interaction of Oct-2 with the basal transcription machinery ('true activation', Croston *et al.*, 1991) or Oct-2 might counteract repressive effects of some

Fig. 3. Activation of promoters by Oct-2 in a fractionated system. (A) κ light-chain promoter plasmid $p\kappa\beta+21$ or REF Δ plasmid driven by an SV40 enhancer element (Picard and Schaffner, 1985) (90 ng) were transcribed in the presence of 0 (lanes 1 and 4), 0.17 (lanes 2 and 5) or 0.34 (lanes 3 and 6) pmol Oct-2 and transcripts were analyzed by RNase protection. (B) Transcription with human TATA binding protein (TBP), TFIID or yeast TBP. Octamer promoter plasmid $p70Zhep^{-}oct^{+}$ (400 ng) was transcribed with saturating amounts of TBP or with TFIID as indicated; 17 pmol Oct-2 was added to even numbered lanes. RNA was analyzed by S1 analysis.

component of the nuclear extracts ('anti-repression'). While complete histone assembly is unlikely in a nuclear extract (cited in Carey *et al.*, 1990), the presence of histone H1 has been documented in *Drosophila* nuclear extracts (Croston *et al.*, 1991). We therefore tested the activity of Oct-2 in a fractionated system utilizing recombinant proteins (TFIIB, TFIIE) and purified fractions (TFIIA, TFIID, TFIIF/H and RNA polymerase). Activation was observed with both a κ light-chain promoter and a composite promoter (Figure 3A and B), indicating that Oct-2 can interact with purified component(s) of the basal transcriptional machinery. While it is possible that trace amounts of a general repressor such as H1 are present in the purified fractions, we consider it unlikely that such a factor is important in Oct-2 activation because, as noted below, Oct-2 functionally acts together with TFIID. Similar activation with Oct-2 is obtained using TFIID purified by three different methods, as well as crude extracts (data not shown). It is possible, however, that Oct-2 may counteract some transcriptional repressors thought to interact directly with TBP (Meisterernst and Roeder, 1991; Inostroza *et al.*, 1992).

Significant activation with Oct-2 is only observed when transcription is driven by TFIID, a fraction which contains both the TATA binding protein (TBP) and associated proteins (TAFs) (Pugh and Tjian, 1992), but not with recombinant TBP alone (Figure 3B). Thus, as has been observed for other transcriptional activators (Pugh and Tjian, 1990), Oct-2 requires 'co-activators' present in the TFIID fraction for activity. We have not yet tested Oct-2 for 'anti-repressor activity' on templates with reconstituted nucleosomes, but most transcription factors tested so far have this activity, including Sp1 (Laybourn and Kadonaga, 1991), which has a glutamine-rich activation domain as do Oct-1 and Oct-2.

To date, it has not been reported whether anti-repression also occurs in transcription reactions driven by TBP, where true activation is absent, due to the lack of coactivating TAFs. If this was the case, then the requirement for coactivators may be a hallmark of true activation.

Oct-2 acts together with TFIID to form the preinitiation complex

Preliminary experiments with nuclear extracts and purified basal factors indicated that in the presence or absence of Oct-2, complexes assembled relatively slowly, with maximal complex assembly requiring over 20 min (data not shown). We took advantage of the relatively slow assembly process to dissect the kinetics of complex formation to ascertain where Oct-2 might function. Basal transcription factors known to associate early during formation of a transcriptionally competent complex (TFIIA, TFIIB or TFIID) were preincubated with the template in the presence or absence of Oct-2, and after preincubation, the remaining factors (TFIIE, TFIIF/H and RNA polymerase II) were added for a brief time before single-round transcriptions were performed. We found that the rate-limiting step of complex formation involved TFIID, Oct-2 and the template DNA. Preincubation of Oct-2 and TFIID resulted in amounts of preinitiation complexes similar to those formed when all basal factors were preincubated with Oct-2 (Figure 4, lanes 2 and 17). Addition of Oct-2 after the preincubation period lead to only low levels of complex formation (lane 3), although DNA binding by Oct-2 was not rate-limiting (< 1 min, data not shown). Thus, Oct-2 must be present during the preincubation with TFIID for high levels of preinitiation complexes to form. Preincubation with TFIIB (Figure 4, lanes 4–6) had no stimulatory effect. The levels of transcription complex formation were not markedly changed when the Oct-2–TFIID reaction also included TFIIA and/or TFIIB (lanes 7–15), indicating that with conditions used here, association of these factors with the complex is probably not rate-limiting. Even though it is possible that there are trace amounts of TFIIB and TFIIA in the TFIID fraction, it is unlikely that they contribute significantly to complex formation, because additional TFIIA and TFIIB must be added for transcription to be detected.

Surprisingly, preincubation of TFIID with TFIIA did not lead to appreciably increased basal transcription levels (lane 1 versus 13), indicating that, at least on this promoter, TFIIA does not appear to stimulate TFIID interaction with the template. [We have also tested a more highly purified TFIIA fraction (Cortes *et al.*, 1992) with the same results.] We did note that preincubation of TFIIA and TFIID enhanced readthrough transcription in multiple-round assays (Figure 5, lane 7), suggesting that TFIIA can facilitate TFIID association with at least one site on the template. This readthrough is not prominent in single-round assays (e.g. Figure 4, lane 13) probably because the elongation time is too brief for transcription through the mapped region.

The functional interaction between Oct-2 and TFIID during the preincubation period required the template. If the DNA was added after the preincubation, together with the additional basal factors, only low levels of preinitiation complexes were obtained (Figure 4B). Transcription obtained by 'late' addition of Oct-2 was reduced in cases when TFIID was first preincubated with the template (compare lanes 6 and 18 with 3, 9, 12 and 15) because of TFIID sequestration, as discussed below.

single-round transcription

Fig. 4. Functional interaction of TFIID and Oct-2 in formation of preinitiation complexes. (A) Preincubation of TFIID and Oct-2 with DNA produces elevated levels of preinitiation complexes. Indicated basal factor(s) were preincubated 20 min with plasmid p70Zhep+oct+ containing an intact heptamer/octamer binding site derived from the 70Z IgH heavy-chain promoter (Kemler *et al.*, 1989) (400 ng) and OVEC-ref (400 ng) in transcription buffer, remaining basal factors were added to allow preinitiation complexes to assemble for 5 min and then single-round transcriptions were carried out. Oct-2 (8.3 pmol) was added (+) at 0 min or at 20 min ('late') as indicated. RNA was analyzed by S1 nuclease protection. Transcription of the reference promoter was undetectable in single-round transcription assays, thus this portion of the gel is not shown. (B) Functional interaction of TFIID and Oct-2 requires template DNA. Lanes 1–3, DNA added at 20 min; lanes 4–6, DNA added at 0 min. (C) Kinetic scheme for single-round transcription assays.

Template commitment by TFIID

Oct-2 might promote TFIID binding to the TATA box, or alternatively, it might interact with TFIID after the factor has bound. To see if Oct-2 affected TFIID binding to the TATA box, we attempted to use DNase I footprinting. Although we could footprint Oct-2 and TBP (data not shown), we were unable to obtain a TFIID footprint, probably because the factor is not abundant enough in the fractions tested. Instead, we used more sensitive functional assays, which indicated that Oct-2 influences DNA binding by TFIID. We noted that if TFIID is allowed to associate with the template in the absence of Oct-2, it becomes intransigent to later stimulation by Oct-2 in multiple-round transcriptions (Figure 5A). Preincubation of TFIID alone (lanes 2 versus 3) or with TFIIA (lanes 8 versus 9) reduced the ability of Oct-2 to activate transcription when added late to the reaction, although preincubation of TFIIA alone did not (lanes 5 versus 6). [A similar effect was noted by Meisterernst *et al.* (1991) but there, loss of TFIID activity was dependent on TFIIA.] The inhibition was also noted in the presence of all factors (lanes 10–12). Significantly, TFIID is stable in the absence of DNA (Figure 4B and data not shown) and loss of activity due to preincubation with

DNA could be overcome by addition of more TFIID at the end of the preincubation (Figure 5B), which indicates that the inhibition is not due to nuclease activity or proteolysis.

We used template challenge assays to determine if the loss of TFIID activity described above was caused by competition for TFIID by other sites on the DNA (Figure 6). A challenge template containing the adenovirus major late promoter TATA box was added to preincubation reactions containing TFIID, Oct-2 and the test promoter. If TFIID was allowed to preincubate with the challenge template before addition of Oct-2 and the test template, the formation of transcription complexes was inhibited (lanes 2 versus 8), mimicking the effect seen in Figure 5A and B. Addition of the challenge template after formation of Oct-2–TFIID–promoter complexes was not inhibitory, indicating that the Oct-2–TFIID complex is resistant to competition (Figure 6, lanes 2 versus 4). Another transcriptional activator, MLTF, has also been observed to stabilize preinitiation complexes to challenge (Carcamo *et al.*, 1989). As observed before, Oct-2 was required for appreciable amounts of complexes to form, thus it was not possible to judge whether TFIID alone was more easily competed than Oct-2–TFIID complexes. We conclude that Oct-2 can influence TFIID–DNA association because in the absence of Oct-2,

Fig. 5. Preincubation of TFIID with DNA prevents subsequent activation of transcription by Oct-2 in multiple-round transcriptions. **(A)** TFIID and/or TFIIA were preincubated with octamer promoter template (p70Zhep⁺oct⁺) (200 ng) and OVEC-ref (200 ng) for 20 min prior to addition of NTPs and remaining basal factors. Multiple rounds of transcription were allowed to occur over 60 min and RNA was mapped by S1 nuclease analysis. Oct-2 (6.7 pmol) was added (+) at 0 min or 20 min ('late'). Preincubation of all factors with or without Oct-2 (lanes 10 and 11) does not yield higher levels of transcription compared with no preincubation (lanes 13 and 14) because multiple rounds of transcription are carried out, unlike the single-round transcription in Figure 4. **(B)** Preincubation of TFIID (270 ng) with template in the absence of Oct-2 (added 'late') gives reduced transcription (lanes 1 and 2); transcription is restored by addition of more TFIID (270 ng) (lane 3). **(C)** Kinetic scheme for multiple-round transcription assays.

TFIID can be 'sequestered', apparently by binding to other sites on the template.

Oct-2 is required continuously at the promoter for reinitiation

If Oct-2 is required solely to facilitate the association of TFIID with the TATA box, then it might be dispensable afterwards, as has been suggested for the ATF activator (Hai *et al.*, 1988). We tested this possibility by examining the effect of removing Oct-2 using competitor oligonucleotides in single- and multiple-round transcriptions. Oct-2 and TFIID were preincubated with the template, and an oligonucleotide containing octamer binding sites, or a control with mutated binding sites, was added prior to initiation of transcription (Figure 7A). In single-round transcriptions, addition of the oligonucleotide at the outset of the incubation completely inhibited the reaction (lane 2 versus 4), while addition after the basal factors had been added, just prior to initiation, had little effect (lane 2 versus 3). In contrast, multiple-round transcriptions were strongly inhibited by the octamer competitor, even after the first round of complex assembly (Figure 7A, lanes 8 versus 9), indicating that Oct-2 is required continuously at the promoter. Reinitiation accounts

for most of the higher (15- to 20-fold) signal seen in multiple-versus single-round transcription, as longer preincubation in single-round transcriptions results in only a modest (2.5-fold) increase (not shown). As expected, addition of the competitor at the beginning of the preincubation (lane 10) reduced transcription to basal level, equivalent to that obtained in the absence of Oct-2 (lane 7). The amount of transcript obtained when the competitor was added after initial complex assembly was higher than that seen in the single-round assays (lanes 3 versus 9), which indicates that a few additional rounds of transcription do occur, presumably due to a time-lag in removal of Oct-2.

Activated versus basal transcription

In multiple-round transcriptions, TBP-driven transcription of octamer promoters is insensitive to the presence of Oct-2, presumably because coactivators are lacking (Figure 3B). The failure of TBP to permit high level transcription is evident in the single-round assay as well (Figure 8A, lanes 11 and 12) which means that the low activity seen with TBP is due to reduced preinitiation complex assembly, rather than a low frequency of reinitiation, for example. (Here, Oct-2 suppressed TBP-driven readthrough transcription, possibly

Fig. 6. (A) Template commitment of TFIID: preincubation of TFIID with challenge template reduces activated complex formation. TFIID was preincubated in transcription buffer; 0.3 pmol Oct-2 and 67 ng octamer promoter plasmid p70Zhep⁺oct⁺ were added at 20 min, basal factors at 40 min and challenge template pG5MLT (adenovirus 2 major late promoter TATA box, 200 ng) (Sawadogo and Roeder, 1985) were added at 0, 20 or 40 min. Single-round transcriptions were carried out and RNA analyzed by S1 nuclease protection. Quantitation of transcripts (relative to lane 2 = 1.0): lane 4, 1.2; lane 6, 0.6; lane 8, 0.35. 'Basal' transcription was too low to quantitate. **(B)** Kinetic scheme for single-round transcription assays.

by a 'roadblock' mechanism.) A titration of TBP demonstrated that the low signal obtained with TBP was not due to limiting amounts of this reagent (Figure 8B). DNase I footprinting (not shown) showed clear protection of the TATA box when using amounts of TBP comparable with that present in transcription reactions. Thus, TBP can bind readily to the TATA box. However, the high level of readthrough transcription from TBP (unlike TFIID) (Figure 8A, lanes 11 and 12, and 8B) indicates that other sites on the template are also efficiently used as promoters and will compete for basal transcription factors. Experiments using the 'G-less cassette' technique (Sawadogo and Roeder, 1985) cannot detect such alternative initiation, causing overall TBP-driven transcription to appear low.

The effect of Oct-2 might be to increase the effective concentration of TFIID at the promoter, thus we tested whether basal transcription could be stimulated simply by increasing the amount of TFIID in single-round transcriptions (Figure 8A, lanes 1–4). Oct-2 stimulated complex formation increased, but basal level transcription remained low. Thus, under the conditions used, Oct-2 is absolutely required for efficient utilization of this promoter.

Oct-2 glutamine domain is required for formation of preinitiation complexes

To characterize which domains of Oct-2 were required for transcriptional activation *in vitro*, mutant proteins lacking portions of the N- or C-termini (characterized earlier *in vivo*) were tested for their ability to activate transcription in nuclear extracts and with the fractionated system (Figures 8A and 9). In accord with results obtained *in vivo* (Müller-Immerglück *et al.*, 1990), the deletion of a glutamine-rich activation domain in the N-terminus led to a severe loss of activity in multiple-round transcriptions. An N-terminal

Fig. 7. (A) Removal of Oct-2 by competitor oligonucleotide inhibits multiple rounds of transcription. TFIID, Oct-2 (0.4 pmol) and octamer promoter plasmid p70Zhep⁺oct⁺ (67 ng) were preincubated in transcription buffer. 1 pmol of a competitor oligonucleotide bearing a heptamer/octamer sequence ('octamer') derived from the Ig 70Z heavy chain promoter or a control oligonucleotide with mutations in the heptamer/octamer binding sites ('control') was added at time indicated. NTPs and Sarkosyl were added to reactions shown in lanes 1–6 at 25 min and single-round transcriptions were stopped at 30 min. NTPs were added to reactions shown in lanes 7–12 at 25 min and multiple-round transcriptions were stopped at 85 min. Quantitation of transcripts (relative to lane 2 = 1.0): lane 3, 1.4; lane 4, 0.1; lane 5, 1.2; lane 6, 0.9; lane 7, 1.3; lane 8, 20; lane 9, 4.4; lane 10, 1.3; lane 11, 13; lane 12, 14. **(B)** Kinetic scheme used in single and multiple-round transcriptions.

deletion (2–99, Müller-Immerglück *et al.*, 1990) not impinging on the glutamine-rich domain had little effect on *in vitro* transcriptional activation (data not shown). The serine/threonine rich C-terminal domain had slight activation activity, while the DNA binding POU domain alone was almost inactive. The Oct-2 truncation derivatives were also tested in single-round transcription assays where they were preincubated with TFIID (Figure 8A, lanes 3–10); the activity in this assay coincided with activity measured in multiple-round transcriptions (Figure 9). Interestingly, *in vivo* the Oct-2 glutamine domain is active only from a promoter position, while other activation domains are active also from distal positions (Seipel *et al.*, 1992).

Although the glutamine domain is needed for efficient preinitiation complex formation, it is not clear that it interacts directly with TFIID. This domain may be involved in contacting other components of the basal transcription machinery in steps subsequent to TFIID binding. We found that TBP binds avidly to Oct-2 via the DNA binding POU domain (data not shown); however, the POU domain itself possesses only very weak activation potential (Figure 8A and 9). Furthermore, Oct-2 does not affect TBP-directed transcription and DNA binding in footprinting assays (Figure 8A and data not shown). Therefore, there is no direct correlation between the ability of Oct-2 to bind TBP *in vitro* and transcriptional activation, unlike findings made by others with the viral Zta protein (Lieberman and Berk, 1991), which may indicate that these activators operate via a distinct mechanism.

Discussion

Studies with the adenovirus 2 major late promoter (AdMLP) have pointed to a sequential process in which basal transcription factors associate with the promoter: first TFIID

Fig. 8. Activity of Oct-2 mutant proteins and of TBP. (A) Lanes 3–10: activity of Oct-2 mutants correlates directly with ability to facilitate preinitiation complex formation in single-round transcriptions. 'Oct-2', wild-type activator; 'C-term', truncation mutation removing N-terminal 159 amino acids including glutamine-rich domain; 'N-term', truncation mutation removing serine/threonine-rich C-terminal 109 amino acids; 'POU domain', Oct-2 DNA binding domain. Lanes 1–4: higher levels of TFIID increase Oct-2 stimulated, but not basal, transcription. Lanes 11 and 12, TBP-driven basal transcription (80 ng TBP). Single-round transcriptions were carried out as indicated in Figure 4C. TFIID or TBP and activator were included in preincubation with octamer promoter template p70Zhep⁺oct⁺. (B) Titration of human TBP indicates that this factor is not limiting (lane 1, 16 ng; lane 2, 80 ng; lane 3, 400 ng).

Fig. 9. Oct-2 glutamine-rich domain is critical for activation in nuclear extracts and in fractionated system. Multiple-round transcriptions were carried out with octamer promoter plasmid p70Zhep⁺oct⁺ (400 ng) and OVEC-ref promoter plasmid (400 ng).

binds the TATA box, assisted by TFIIA, then TFIIB binds this DA complex, and the DAB complex is recognized by Pol II–TFIIF. Factors TFIIE, TFIIF and TFIIF associate and conformational changes occur prior to initiation (reviewed in Zawel and Reinberg, 1992). Basal factors TFIIG and an initiator box binding factor have also been identified (Garfinkel *et al.*, 1990; Sumimoto *et al.*, 1990; Roy *et al.*, 1991). Although our understanding is far from complete, studies on natural and chimeric transcriptional activators have suggested these factors can act either indirectly, by relieving histone repression (Croston *et al.*, 1991; Workman *et al.*, 1991), and/or directly by facilitating association of TFIID and/or TFIIB with the nascent preinitiation complex (Abmayr *et al.*, 1988; Hai *et al.*, 1988; Horikoshi *et al.*, 1988a,b; Stringer *et al.*, 1990; Lin and Green, 1992; Sundseth and Hansen, 1992). Our studies

indicate that Oct-2 activates transcription by direct interaction with the basal machinery, and that the glutamine-rich activation domain found previously to be important for activity *in vivo* is required for assembly of preinitiation promoter complexes.

Oct-2 acts with TFIID to establish a preinitiation complex

Using an *in vitro* system, we determined that Oct-2 stimulated transcription of octamer promoters (Figure 1A and B) by facilitating formation of preinitiation complexes (Figure 2). Oct-2 also stimulated transcription in a fractionated system (Figure 3A and B), showing that it interacts directly with components of the basal transcription machinery, and is thus a 'true activator'. Activation was dependent upon 'coactivators'—components of the TFIID

fraction distinct from TBP (Figure 3B). We dissected the kinetics of preinitiation complex formation, and found that the rate-limiting step involved an interaction of TFIID with the DNA that was dependent on Oct-2 (Figure 4). A similar result was obtained with an octamer–AdMLP TATA box construct, indicating that this interaction was not particular to the β globin TATA box (data not shown). If Oct-2 was not included in the preincubation, TFIID was no longer capable of supporting specific preinitiation complex formation, apparently because of being sequestered by binding to alternate sites on the template (Figure 5A). However, once formed, DNA–Oct-2–TFIID complexes were resistant to challenge (Figure 6).

Complex formation was not facilitated by including TFIIB and/or TFIIA in the preincubation with Oct-2 and TFIID (Figure 4), therefore interaction of these factors with the complex must occur rapidly. It is possible that Oct-2 interacts with TFIIB, but the interaction would only be important in the context of TFIID, and not TBP, since Oct-2 is not required for TBP-driven transcription from this promoter. If TFIIB is present in the nucleus in limiting amounts, as it seems to be in nuclear extracts (Lin and Green, 1991; Sundseth and Hansen, 1992), direct or indirect binding of this factor might be an important step in facilitating preinitiation complex assembly. However, limiting levels of TFIIB are clearly not the only impediment to efficient transcription, since our system employed recombinant TFIIB in saturating amounts (see also White *et al.*, 1992).

Although transcription was dependent upon TFIIA, inclusion of the fraction was not necessary in the preincubation, and in the absence of Oct-2, preincubation of TFIIA with TFIID did not stimulate complex formation at the octamer promoter. TFIIA appears to have a function other than stabilizing association of TFIID with this promoter; Cortes *et al.* (1992) suggest that TFIIA may counteract negative regulatory molecules which are present in the TFIID fraction. Consistent with this model, TFIIA is not required for basal transcription of the template with TBP (Cortes *et al.*, 1992, and our unpublished data).

No 'hit-and-run' activation: Oct-2 is required continuously at the promoter for reinitiation

In order to elucidate further the role of Oct-2 in promoter activation, we tested the effect on transcription of removing Oct-2 from the promoter by addition of excess competitor oligonucleotide. Kinetic analysis using single-round transcriptions (Figure 7A) revealed that fully assembled preinitiation complexes are resistant to such competition, suggesting that either Oct-2 is no longer needed after the complex is fully assembled, or that it is no longer accessible to competition. In contrast to single-round transcriptions, multiple-round transcriptions were strongly inhibited by the oligonucleotide. Oct-2 may be required for maintaining TFIID in an 'active' conformation or for binding of another factor that dissociates from the promoter after each round of initiation. Alternatively, removal of Oct-2 may cause TFIID to dissociate, although this seems unlikely; both TBP and TFIID dissociate slowly from DNA (Nakajima *et al.*, 1988; Schmidt *et al.*, 1989).

Our results with Oct-2 stand in apparent contrast to those obtained with the transcription factor ATF. Stimulation of the adenovirus E4 promoter was only mildly inhibited by

removal of ATF after preinitiation complex formation (Hai *et al.*, 1988), and it was suggested that once the TFIID complex was assembled, the activator might be dispensable. However, only a low rate of reinitiation was obtained in this system, so it was difficult to measure conclusively effects on reinitiation. Other *in vitro* studies have suggested that activators do play a role in reinitiation; White *et al.* (1992) showed that antibody against the VP16 moiety of a chimeric activator inhibited reinitiation, but it was not possible to distinguish between blocking of a VP16 activity and non-specific steric hindrance by the promoter-bound antibody. Carcamo *et al.* (1989) found MLTF stimulation was greater in the absence of a detergent block, and concluded that MLTF stimulates transcription in part by facilitating reinitiation. Neither of these studies involved removal of the activator from the promoter, however. Our findings with Oct-2 have also been presaged by *in vivo* studies: *in vivo* footprinting showed that transcription of the tyrosine aminotransferase gene is tightly coupled to binding of transcriptional activators. Loss of activator binding closely parallels cessation of gene transcription (Reik *et al.*, 1991). In addition, while it was originally thought that immunoglobulin promoters could be locked in an 'on' position, such that transcription would continue even after deletion of the enhancer, it was found that a more distant enhancer was responsible for this continued promoter activity (as discussed in Schaffner, 1988, and references therein). Thus, it seems likely that transcription of a gene involves the continuous action of transcriptional activators.

What is 'basal' transcription? TBP versus TFIID

The term 'basal' transcription has been applied both to activity seen with TFIID in the absence of 'upstream' activators and to activity of TBP, which does not respond to activators. Why is TBP incapable of giving higher level transcription? We determined by footprinting (not shown) that the amount of TBP used in the transcription reactions was sufficient to protect the TATA box completely. Therefore, either TBP is inefficient in forming preinitiation complexes or TBP complexes elsewhere on the template compete for basal factors, reducing overall transcription from the octamer promoter. This latter possibility seems likely since TBP can readily drive 'readthrough' transcription from cryptic promoter sites (Figure 8A and B). As TBP is added to saturating levels, octamer promoter and upstream readthrough transcription increase in parallel. TFIID-driven initiation, on the other hand, appears to be more selective and is dependent on an activator to form an active preinitiation complex (Figure 8A, lanes 1–4). Thus, the difference between 'basal' transcription with TBP and 'activated' transcription with TFIID might be a reflection of these factors' functional specificity; functional TFIID complexes are concentrated at the target promoter, while TBP complexes are dispersed. While TFIID appears also to associate with non-promoter sites *in vitro*, the lack of an activator binding site nearby probably prevents a functional preinitiation complex from forming. It has been shown recently that TFIID plays a critical role in transcription of promoters lacking a consensus TATA box (Pugh and Tjian, 1992). Thus, specificity of transcriptional initiation seems to be dictated not only by TFIID–DNA (TATA box) interactions, but also by 'upstream' activators, such as Oct-2, which allow successful assembly of preinitiation complexes.

Materials and methods

Preparation of nuclear extracts

HeLa S3 cells were maintained in spinner cultures in Joklik's MEM with 5% fetal calf serum (FCS) to 0.5×10^6 cells/ml. BJA-B (human Burkitt's lymphoma) B-lymphocyte cells were cultured in RPMI 1640 medium with 10% FCS to 1×10^6 cells/ml. Nuclear extracts were prepared from freshly harvested cells essentially as previously described (Westin *et al.*, 1987). After ammonium sulfate precipitation, nuclear proteins were resuspended in buffer containing 20 mM HEPES KOH, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 2 mM $MgCl_2$ and 0.5 mM DTT, aliquoted and stored in liquid nitrogen.

Purification of basal transcription factors

Recombinant human and yeast TBP were produced and purified as described in Maldonado *et al.* (1990) and Cortes *et al.* (1992). 40 ng of S-Sepharose fraction yTBP and 80 ng of phosphocellulose fraction hTBP were used in transcriptions. Recombinant TFIIE was produced and purified as described in Peterson *et al.* (1991). 30 ng of Sephacryl 200 fraction was used per reaction. TFIIB was produced and purified as described in Ha *et al.* (1991). 10 ng of phosphocellulose fraction was used in transcription reactions. Factors from HeLa cells: TFIIA (containing TFIIF) was fractionated as described in Maldonado *et al.* (1990); 12 μ g was used per reaction. TFIID was purified as described in Maldonado *et al.* (1990); 270 ng (3 μ l) of DEA-sephadex fraction was used per reaction. TFIIF/H were fractionated as described in Flores *et al.* (1989). 400 ng of DEA 5PW fraction was used in transcriptions. RNA polymerase II was purified as described in Lu *et al.* (1991). 110 ng of DEA 5PW fraction was used in transcriptions.

Overexpression and purification of Oct-2 and derivatives

Expression vectors. Oct-2 and truncation mutants of Oct-2 were subcloned from eukaryotic expression vectors (Müller-Immerglück *et al.*, 1990) into a modified pET3c (Studier *et al.*, 1990) vector (termed pT7BX) containing a T7 RNA polymerase promoter. The pT7BX vector was constructed by destroying the *Xba*I site at +26 (cleavage, filling in and religation) and ligating a double-stranded oligonucleotide (5' GATCGGATCCCATAGTCTAGA 3' and 5' GATCTCTAGACTTATGGGACCT 3') containing complementary overhanging ends into the *Bam*HI site at +102. *Bam*HI–*Xba*I fragments containing Oct-2 constructs were cloned into the corresponding sites created by this modification. Overexpressed proteins contained amino acids MASMTGGQMQMR at the N-terminus deriving from the T7 gene 10 and the adaptor oligonucleotide. Full-length Oct-2 (amino acids 2–479), a deletion removing the C-terminus (leaving 2–370) and a deletion removing the N-terminus (leaving 161–479) (Müller-Immerglück *et al.*, 1990) expression vectors were prepared. The POU domain vector was prepared by total synthesis of the coding region (Li-Cen Xu, unpublished data) (amino acids 194–356) and ligation of this into the pET3c vector at the *Nde*I (initiation codon) site. In this construct, both cysteine codons have been mutated to serine codons; however, protein from this construct had the same DNA binding, footprinting, activation activity as the wild-type POU domain (194–357) (not shown).

Expression and purification. Expression constructs were transformed into *E. coli* BL21 (DE3), a strain containing the T7 RNA polymerase gene under the control of the lac promoter (Studier *et al.*, 1990). Fresh overnight transformants were grown at 37°C to OD₆₀₀ 0.5 in Luria broth supplemented with 50 μ g/ml ampicillin, induced with 0.4 mM IPTG and grown for 1–2 h. All proteins were highly expressed, and most were found to be insoluble. Oct-2 protein and derivatives were prepared from frozen bacteria by sonication in bacterial lysis buffer (50 mM Tris–Cl pH 8, 100 mM NaCl, 1 mM DTT, 1 mM PMSF, 1 mM EDTA). Insoluble material was separated by centrifugation, treated with lysis buffer containing 6 M urea, incubated 1 h at room temperature, and centrifuged, at which point most of the recombinant protein was soluble. After dialysis to remove urea, the proteins were chromatographed over an octamer DNA affinity column (Kadonaga and Tjian, 1986), eluted with buffer containing 300 mM NaCl and analyzed by SDS–PAGE. The proteins were estimated to be >95% pure and were tested for activity in bandshift, footprint and transcription assays. Oct-2 purified to a similar degree over a BioRex 70 cation exchange column was used in most assays; this fraction had the same activity as that purified on the DNA affinity matrix. Protein concentrations were measured by the Bradford assay (Bradford, 1976) using bovine serum albumin as the standard.

Transcription assays

Nuclear extracts. Transcription reactions (15 μ l) contained 10 mM HEPES KOH, pH 7.9, 10% glycerol, 20 mM KCl, 4 mM $MgCl_2$, 4 mM

spermidine, 0.1 mM EDTA, 0.25 mM DTT, 5 mM creatine phosphate, 0.5 mM each NTP, 3 units RNasin (Promega) and 400 ng each of test and reference template. 15–30 μ g of nuclear extract were added to initiate reactions, which were carried out at 30°C for 60 min. Titrations of Oct-2 indicated that activation was optimal between 1 and 8 pmol protein (5–40 Oct-2 molecules/plasmid); however, no inhibition of transcription was observed at higher levels of activator. RNA was purified and analyzed by S1 nuclease or RNase protection as described (Westin *et al.*, 1987; Kemler *et al.*, 1991).

Fractionated system. Transcription reactions (20 or 40 μ l) contained 20 mM HEPES, pH 7.9, 8 mM $MgCl_2$, 2% PEG, 5.5 mM $(NH_4)_2SO_4$, 5 mM creatine phosphate, 0.5 mM each NTP, 3 units RNasin, 67–400 ng of test template and 400 ng reference template when added. Activation was optimal at Oct-2:plasmid ratios of 10:1, although more Oct-2 was not inhibitory. We routinely observed a higher level of transcriptional activity with nuclear extracts than with fractionated factors, which is likely due to a higher concentration of basal transcription factors (e.g. limiting TFIID in Figure 8A, lanes 1–4). Additional factors may also be lacking in the fractionated system (Meisterernst *et al.*, 1991).

Single-round transcription assays

Nuclear extract or factors were preincubated with template for 20 min, NTPs and Sarkosyl (0.06% with nuclear extract, 0.02% with purified factors) were added, preinitiation complexes were allowed to elongate (usually 5 min) and transcriptions were terminated by addition of 10 μ l 8 M urea, 20 mM EDTA and 2% SDS. Single-round transcriptions performed with purified factors also included a 5 min incubation with the remaining basal factors after the preincubation to permit formation of complete preinitiation complexes prior to addition of detergent and NTPs. No transcripts were seen in the absence of NTPs, indicating that the extracts and fractions were not contaminated with NTPs.

Oligonucleotide competitions

To compete for Oct-2, a double-stranded oligonucleotide containing a consensus heptamer/octamer site derived from the 70Z heavy chain promoter capable of binding two molecules of Oct-2 (Kemler *et al.*, 1989) (5' CGA-GTATCCTGCTCATGAATATGCAAATCCTCTGG 3' and 5' TCGAC-CAGAGGATTTGCATATTCATGAGCAGGATACTCGAGCT 3') or a control oligonucleotide (5' CGAGTATCCTGCTCCGTAATATCAGTC-GCCTCTGG 3' and 5' TCGACCAGAGGCGACTGATATTACGGAG-CAGGATACTCGAGCT 3') was added to the transcription reaction. Oligonucleotides were quantitated first by A260 to ensure that identical amounts of DNA were added.

Quantitation. RNA produced *in vitro* was quantitated by excision of bands from gels followed by Cerenkov counting, or by phosphorescence quantitation with a PhosphorImager.

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References

- Abmayr, S.M., Workman, J.L. and Roeder, R.G. (1988) *Genes Dev.*, **2**, 542–553.
- Annweiler, A., Müller-Immerglück, M.M. and Wirth, T. (1992) *Mol. Cell. Biol.*, **12**, 3107–3116.
- Bradford, M. (1976) *Anal. Biochem.*, **72**, 248.
- Bunick, D., Zandomeni, R., Ackerman, S. and Weinmann, R. (1982) *Cell*, **29**, 877–886.
- Carbon, P., Murgo, S., Ebel, J.P., Krol, A., Tebb, G. and Mattaj, J.W. (1987) *Cell*, **51**, 71–79.
- Carcamo, J., Lobos, S., Merino, A., Buckbinder, L., Weinmann, R., Natarajan, V. and Reinberg, D. (1989) *J. Biol. Chem.*, **264**, 7704–7714.
- Carey, M., Leatherwood, J. and Ptashne, M. (1990) *Nature*, **247**, 710–712.

- Clerc, R.G., Corcoran, C.M., LeBowitz, J.H., Baltimore, D. and Sharp, P.A. (1988) *Genes Dev.*, **2**, 1570–1581.
- Cortes, P., Flores, O. and Reinberg, D. (1992) *Mol. Cell. Biol.*, **12**, 413–421.
- Croston, G.E., Kerrigan, L.A., Lira, L.M., Marshak, D.R. and Kadonaga, J.T. (1991) *Science*, **251**, 643–649.
- Flores, O., Maldonado, E. and Reinberg, D. (1989) *J. Biol. Chem.*, **264**, 8913–8921.
- Garfinkel, S., Thompson, J.A., Jacob, W.F., Cohen, R. and Safer, B. (1990) *J. Biol. Chem.*, **265**, 10309–10319.
- Gerster, T., Balmaceda, C.-G. and Roeder, R.G. (1990) *EMBO J.*, **9**, 1635–1643.
- Ha, I., Lane, W.S. and Reinberg, D. (1991) *Nature*, **352**, 698–695.
- Hai, T., Horikoshi, M., Roeder, R. and Green, M. (1988) *Cell*, **54**, 1043–1051.
- Hawley, D. and Roeder, R. (1989) *J. Biol. Chem.*, **262**, 3452–3461.
- Horikoshi, M., Hai, T., Lin, Y.-S., Green, M.R. and Roeder, R.G. (1988a) *Cell*, **54**, 1033–1042.
- Horikoshi, M., Carey, M.F., Kakidani, H. and Roeder, R.G. (1988b) *Cell*, **54**, 665–669.
- Inostroza, J.A., Mermelstein, F.H., Ha, J., Lane, W.S. and Reinberg, D. (1992) *Cell*, **70**, 477–489.
- Johnson, D., Carayannopoulos, L., Capra, J., Tucker, P. and Hanke, J.H. (1990) *Mol. Cell. Biol.*, **10**, 982–990.
- Kadonaga, J.T. and Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 5889–5893.
- Kemler, I., Schreiber, E., Müller, M.M., Matthias, P. and Schaffner, W. (1989) *EMBO J.*, **8**, 2001–2008.
- Kemler, I. and Schaffner, W. (1990) *FASEB J.*, **4**, 1444–1449.
- Kemler, I., Bucher, E., Seipel, K., Müller, M.M. and Schaffner, W. (1991) *Nucleic Acids Res.*, **19**, 237–242.
- Kerppola, T.K. and Kane, C. (1991) *FASEB J.*, **5**, 2833–2842.
- Ko, H.-S., Fast, P., McBride, W. and Staudt, L.M. (1988) *Cell*, **55**, 135–144.
- LaBella, F., Sive, H.L., Roeder, R.G. and Heintz, N. (1988) *Genes Dev.*, **2**, 32–39.
- Laybourn, P.J. and Kadonaga, J. (1991) *Science*, **254**, 238–245.
- LeBowitz, J.H., Kobayashi, T., Staudt, L., Baltimore, D. and Sharp, P.A. (1988) *Genes Dev.*, **2**, 1227–1237.
- Lieberman, P.M. and Berk, A.J. (1991) *Genes Dev.*, **5**, 2441–2454.
- Lin, Y.-S. and Green, M. (1991) *Cell*, **64**, 971–981.
- Lin, Y.-S., Ha, I., Maldonado, E., Reinberg, D. and Green, M.R. (1991) *Nature*, **353**, 569–571.
- Lu, H., Flores, O., Weinmann, R. and Reinberg, D. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 10004–10008.
- Maldonado, E., Ha, I., Cortes, P., Weis, L. and Reinberg, D. (1990) *Mol. Cell. Biol.*, **10**, 6335–6347.
- Meisterernst, M., Roy, A.L., Lieu, H.M. and Roeder, R.G. (1991) *Cell*, **66**, 981–993.
- Mitchell, P. and Tjian, R. (1989) *Science*, **245**, 371–378.
- Müller, M.M., Ruppert, S., Schaffner, W. and Matthias, P. (1988) *Nature*, **336**, 544–551.
- Müller-Immerglück, M.M., Schaffner, W. and Matthias, P. (1990) *EMBO J.*, **9**, 1625–1634.
- Nakajima, N., Horikoshi, M. and Roeder, R.G. (1988) *Mol. Cell. Biol.*, **8**, 4028–4040.
- Peterson, M.G., Inostroza, J., Maxon, M.E., Flores, O., Admon, A., Reinberg, D. and Tjian, R. (1991) *Nature*, **354**, 369–373.
- Picard, D. and Schaffner, W. (1985) *EMBO J.*, **4**, 2831–2838.
- Pierani, A., Heguy, A., Fujii, H. and Roeder, R.G. (1990) *Mol. Cell. Biol.*, **10**, 6204–6215.
- Pugh, B.F. and Tjian, R. (1990) *Cell*, **61**, 1187–1197.
- Pugh, B.F. and Tjian, R. (1992) *J. Biol. Chem.*, **267**, 679–682.
- Reik, A., Schütz, G. and Stewart, A.F. (1991) *EMBO J.*, **10**, 2569–2576.
- Roeder, R.G. (1991) *Trends Biochem. Sci.*, **16**, 402–408.
- Roy, A.L., Meisterernst, M., Pognonec, P. and Roeder, R.G. (1991) *Nature*, **354**, 245–248.
- Sawadogo, M. and Roeder, R.G. (1984) *J. Biol. Chem.*, **259**, 5321–5326.
- Sawadogo, M. and Roeder, R.G. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4394–4398.
- Schaffner, W. (1988) *Nature*, **336**, 427–428.
- Scheidereit, C., Cromlish, J.A., Gerster, T., Kawakami, K., Balmaceda, C.-G., Currie, R.A. and Roeder, R.G. (1988) *Nature*, **336**, 551–557.
- Schmidt, M.C., Zhou, Q. and Berk, A.J. (1989) *Mol. Cell. Biol.*, **9**, 3299–3307.
- Schöler, H.R. (1991) *Trends Genet.*, **7**, 323–329.
- Schreiber, E., Harshman, K., Kemler, I., Malipiero, U., Schaffner, W. and Fontana, A. (1990) *Nucleic Acids Res.*, **18**, 5495–5503.
- Seipel, K., Georgiev, O. and Schaffner, W. (1992) *EMBO J.*, in press.
- Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) *Methods Enzymol.*, **185**, 60–89.
- Stringer, K.F., Ingles, C.J. and Greenblatt, J. (1990) *Nature*, **345**, 783–786.
- Sturm, R.A., Das, G. and Herr, W. (1988) *Genes Dev.*, **2**, 1582–1599.
- Sumimoto, H., Ohkuma, Y., Yamamoto, T., Horikoshi, M. and Roeder, R.G. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 9158–9162.
- Sundseth, R. and Hansen, U. (1992) *J. Biol. Chem.*, **267**, 7845–7855.
- Tanaka, M. and Herr, W. (1990) *Cell*, **60**, 375–386.
- Tanaka, M., Lai, J.-S. and Herr, W. (1992) *Cell*, **68**, 755–767.
- Westin, G., Gerster, T., Müller, M.M., Schaffner, G. and Schaffner, W. (1987) *Nucleic Acids Res.*, **15**, 6787–6798.
- White, J., Brou, C., Wu, J., Lutz, Y., Moncollin, V. and Chambon, P. (1992) *EMBO J.*, **11**, 2229–2240.
- Workman, J.L., Taylor, I.C.A. and Kingston, R.E. (1991) *Cell*, **64**, 533–544.
- Yang, J., Müller-Immerglück, M.M., Seipel, K., Jansen, L., Westin, G., Schaffner, W. and Pettersson, U. (1991) *EMBO J.*, **10**, 2291–2296.
- Zawel, L. and Reinberg, D. (1992) *Prog. Nucleic Acids Res. Mol. Biol.*, **45**, in press.
- Zeleznik-Le, N.J., Itoh-Lindstrom, Y., Clarke, J.B., Moore, T.L. and Ting, J.P.-Y. (1992) *J. Biol. Chem.*, **267**, 7677–7682.

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