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Interaction of Steroid Hormone Receptors with the Transcription Initiation Complex

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I. Introduction

REGULATION of gene expression by steroid hormones is a traditional field of molecular endocrinology. The hormones act by binding to intracellular receptors, which themselves orchestrate the transcriptional response. After the cloning of the hormone receptors and the identification of their target sequences on DNA at the end of the 1980s, the question of how steroid hormones modulate the activity of various genes in target cells seemed to be solved. It was clear that steroid hormone receptors are ligand-activated transcriptional modulators, which in most cases act through binding to specific sequences on DNA called hormone-responsive elements (HREs) (1). Careful mutational analysis identified a modular structure of the receptors composed of a DNA-binding domain, nuclear localization signals, a ligand-binding domain, and several transactivation domains (2). Interactions of the receptors with components of the basal transcriptional machinery and with sequence-specific transcription factors were assumed to mediate their transcriptional effects. The prevalent opinion was that the mechanism of action of steroid hormones was a closed chapter ready for the textbooks and what remained to be done to complete the picture was merely the collection of details. Many of the leading laboratories in the field moved on to the study of what seemed to be more relevant questions, such as the role of the receptors in mouse physiology and development, as deduced from the targeted disruption of the receptor genes. However, the initial burst of data represented just a catalogue of some of the components of the system, and the question of how the hormone-receptor complex really does regulate transcriptional efficiency in the intact cells remained wide open. Hence, although the steroid receptors were among the first transcription factors from higher eukaryotes to be identified and studied in detail, the first round of results did not add essential new insights into eukaryotic-specific regulatory mechanisms.

On the other hand, in the last 5 yr our knowledge of transcriptional control in animal cells has been vastly increased with the revelation of eukaryote-specific mechanisms. Indeed more polypeptides involved in eukaryotic transcription initiation have been identified in the past 5 yr than in all the previous history of the field. This allows a reformulation of the mechanisms of transcriptional control by steroid hormone receptors on a much more solid basis. In revising the wealth of recent literature we will focus on transcription initiation and will deliberately omit many aspects of receptor biology not directly involved in transcriptional control, *e.g.* their interactions with heat shock proteins and the cross-talk with other signaling pathways acting at the level of the cell membrane. Although these interactions may ultimately impinge on the transcriptional properties of the receptors, these effects are indirect, and a detailed description would deviate from the main focus of this review. Given

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the unfeasibility of reviewing the enormous amount of literature in the field of transcriptional control, we will illustrate the general mechanisms by selecting just a few well characterized examples and will have to leave unmentioned many other interesting systems. Of course, our choices will be influenced by our own experimental work, and we apologize in advance to those colleagues whose work is not mentioned. In particular, we will not mention the transcriptional repression observed with unliganded retinoic and thyroid hormone receptors (3, 4), as there is no evidence for such a mechanism in transcriptional regulation by steroid hormone receptors. Our main interest will be directed to the biochemical aspects of transcriptional initiation relevant to hormone receptor interactions, but we will also refer to the knowledge gained by genetic means on the role of chromatin in transcriptional control particularly in yeast. Finally, we will indicate important questions to be addressed in the future.

II. Assembly of the Transcription Initiation Complex

Before describing the interactions of steroid hormone receptors with the general transcriptional machinery, we will attempt to summarize the state of our knowledge on transcription initiation in animal cells. In eukaryotic cell nuclei, there are three different RNA polymerases, I, II and III, each dedicated to the transcription of different sets of genes with characteristic promoters. These promoters are recognized by two types of transcription factors: the basal or general transcription factors (GTFs), which interact with the core promoter elements, and the modulator or sequence-specific transcription factors, which generally interact with sequences located further upstream. GTFs are sufficient to determine RNA polymerase specificity and to direct low levels of transcription, whereas the sequence-specific transactivators act by enhancing or reducing the basal level of transcription. We will concentrate on the transcription by RNA polymerase II (RNAP-II) of TATA box-containing promoters, which are the vast majority of hormonally regulated promoters. RNAP-II promoters can be divided into two large classes: the mRNA and the small nuclear (sn) RNA promoters. The mRNA promoters can be divided again into two classes depending on whether or not they contain a TATA box. The TATA box-containing mRNA promoters may consist of either the TATA box alone or the TATA box and an initiator sequence (Inr) that spans the transcriptional start site. The TATA-less mRNA promoters consist of just the Inr.

Although it is a central player, the catalyzing enzyme RNAP-II itself has not been studied in as much detail as many of the GTFs. The yeast enzyme is composed of 12 subunits, all of which have been cloned (5), but the subunits of the mammalian enzyme are not all available (6). RNAP-II interacts with many GTFs, in particular through the C-terminal repeat domain (CTD) of its largest subunit, the length of which is variable among different species (7).

Most of the factors involved in formation of the transcription initiation complex have been identified and cloned (Table 1). In addition to RNAP-II, these include TFIID, TFIIB, TFIIA, TFIIF, TFIIE, and TFIIH (for a review see Ref. 8). In describing the assembly of the transcription initiation complex, one could distinguish between the following: nucleation by binding of TFIID, binding of GTFs connecting to RNAP-II, and actual recruitment of the polymerase (9). However, for convenience we will distinguish between the so-called minimal transcription system, which is able to perform just basal transcription, and the more efficient complete transcription systems that are able to respond to addition of sequence-specific transactivators (8). One should keep in mind that this distinction is based mainly on the results of cell-free transcription assays, and its physiological relevance remains debatable. In addition to the assembly of the transcription initiation complex, promoter clearance is also a step relevant to the control of transcriptional efficiency and may required additional polypeptides as well as phosphorylation of RNAP-II. Whether the assembly of the different forms of the transcriptional initiation complex is catalyzed by sequential addition of factors to a basal transcription complex or, rather, by recruitment of complexes assembled through different pathways remains an open and important question (Fig. 1).

A. Minimal transcription system: RNAP-II, TATA box-binding protein (TBP), TFIIB, and TFIIFβ

Although nowadays the concept of preformed holoenzyme complexes able to perform efficient transcription is gaining momentum (see below), the prevailing idea is that the preinitiation complex is assembled in an ordered stepwise fashion, with binding of TFIID to the TATA box as the first step, followed by sequential binding of TFIIB, RNAP-II,

TABLE 1. General transcription factors

Factor	M_r (kDa)	Interactions	Function
TBP[a]	38	DNA, TAFIIs, TFIIB RNAP-IIA, TFIIE, transactivators	Binding to TATA box, bending of DNA
TFIIB	33	DNA, RNAP-II, TBP TFIIFβ, transactivators	Recruitment of RNAP-II/TFIIF mediates transactivation
TFIIA	α 35, β 19, γ 12	TBP, Dr1, Dr2 transactivators	Modulates TFIID binding, mediates transactivation
TFIIF	α 74, β 30 (RAP74, RAP30)	RNAP-II, TFIIB	Recruitment of RNAP-II, prevents pausing
TFIIE	α_2 56, β_2 34	RNAP-IIA, TBP, $TFIIF_\alpha$, $TFII_\beta$, TFIIH, transactivators	Recruitment of RNAP-IIA and TFIIH promoter clearance, DNA repair
TFIIH	89, 80, 65, 50 44, 41, 38, 34	DNA, TFIIE	CTD kinase, promoter release ATPase, helicase, DNA repair

[a] For TFIID, see Table 2.

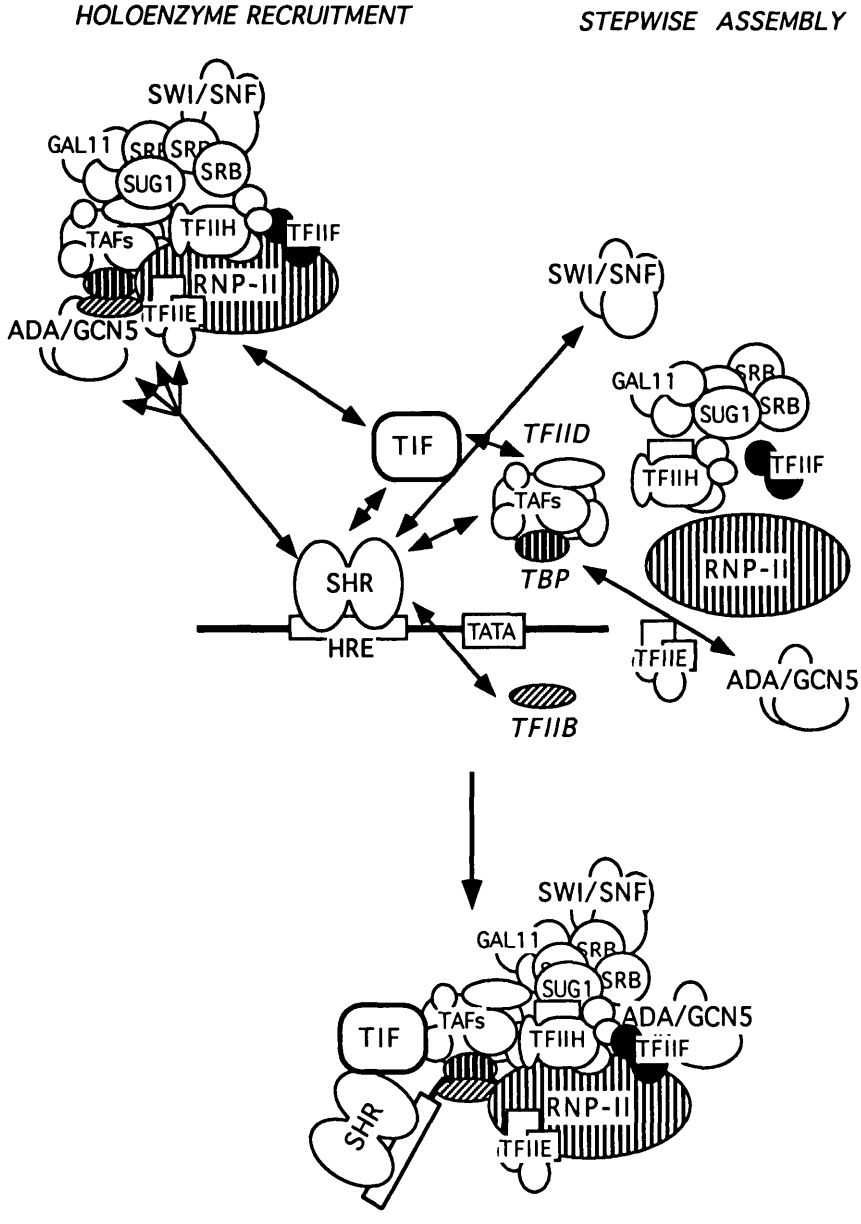

FIG. 1. Assembly of the transcription initiation complex on a hormone responsive minimal promoter. Two alternative pathways are depicted. On the *left*, the recruitment of the complete RNAP-II holoenzyme; on the *right*, the stepwise assembly of the individual components. In both pathways interactions with the receptors (symbolized by *arrows*) could be direct or mediated by one or multiple coactivators (TIF). The SWI/SNF complex and the ADA/GCN5 complex are possible complexes of the holoenzyme complex involved in chromatin transcription (213, 258).

and TFIIF, to build the minimal transcription initiation complex (8).

TFIID is composed of the TBP and TBP-associated factors (TAF_{II}s), forming several complexes, the dominant one of about 750 kDa. The designation of the main component of TFIID as TBP is misleading as both the TATA box and the Inr appear to recruit the TBP-containing complex TFIID. TBP is also required for transcription by all three eukaryotic RNAPs (10, 11) and is part of the initiation complex SL1 formed on the TATA-less ribosomal RNA promoters transcribed by RNAP-I and of the TFIIIB complex formed on some RNAP-III promoters.

TBP is a highly conserved protein, particularly at its C-terminal half, which encompasses two DNA-binding direct repeats of 66–67 amino acids each flanked by a basic region (12). Its overall shape is saddle-like, and the concave surface of the saddle binds to the minor groove of DNA over the region of the TATA box. Binding induces a drastic bend of the DNA (13–15), which seems to be an important component of the site-specific recognition by TBP (16, 17). Binding to the TATA region also induces conformational changes in the N-terminal domain of the protein and stabilizes the C-terminal core domain in partial proteolysis experiments (18). Thus, at the very initial step in transcription there is already a bidirectional communication between proteins and DNA.

TBP contacts RNAP-II at the CTD of its largest subunit. The domain consists of a repeated heptad motif, the length of which varies between 26 copies in yeast and 52 copies in mouse, that can be highly phosphorylated. Significantly, TBP interacts only with the nonphosphorylated form of RNAP-II, RNAP-IIA (19), which is the form known to enter the initiation complex (20).

Using supercoiled templates, basal transcription of several promoters, including the adenovirus major late promoter,

does not require TFIID but can proceed with just recombinant TBP, TFIIB, the 30-kDa subunit of TFIIF ($TFIIF_\beta$), and RNAP-II (21). In the TATA-less promoter of the adenovirus associated virus AAV-P5, initiation can take place in the absence of TBP, with only TFIIB and RNAP-II, but in which case binding of the transcription factor YY1 to the initiator region is required (22). TFIIB, a 33-kDa protein with affinity for single-stranded DNA (23), contacts DNA upstream and downstream of the TATA box on the concave side of the strong bend induced by TBP binding, suggesting that TBP may play an architectural role in the assembly of the preinitiation complex (24–26). TFIIB also contacts TBP, $TFIIF_\beta$, the CTD of RNAP-II (19, 27), and, as we will see, several transactivators (28, 29). Different domains of TFIIB are involved in these interactions (30). The CTD, homologous to TBP and prokaryotic σ-factors, is folded into a compact, protease-resistant core, which binds to promoter-TBP complexes (31) but is transcriptionally inactive (32). The N-terminal region of TFIIB is required for binding to $TFIIF_\beta$ and to the CTD of RNAP-II (27, 32). Thus, TFIIB could be instrumental for the recruitment of RNAP-II complexed to TFIIF and could eventually even determine the site of initiation. These results show that basal transcription can be accomplished *in vitro* with only three GTFs in addition to RNAP-IIA.

TFIIF mediates the association of RNAP-II with promoter sequences containing transcription factors IID and IIB (33). The resulting DNA-protein complex contains RNAP-II and the two subunits of TFIIF (α and β), but the cloned small subunit, $TFIIF_\beta$, is sufficient for the recruitment of polymerase II to the complex, through interactions with the enzyme and with TFIIB. This ability of $TFIIF_\beta$ to recruit RNAP-II to a promoter resembles the function of σ-factors in prokaryotes (34). The large subunit, $TFIIF_\alpha$, is not essential for minimal transcription of many promoters but receives signals from sequence-specific transactivators, *i.e.* the serum-responsive factor (35). $TFIIF_\beta$ not only assists TFIIB in recruiting RNAP-II but also increases the elongation rate by suppressing polymerase pausing (33). Whereas the initiation activity is mediated by the CTD, including the DNA-binding domain homologous to bacterial σ-factors, the elongation function is mediated by sequences further upstream, which bind RNAP-II, and by the N-terminal half of the molecule (33).

B. Complete transcription system: TFIIA, TFIIE, and TFIIH

Addition to the minimal TBP-containing transcription system of TFIIE, a tetramer with two different subunits, and the 74-kDa subunit of TFIIF, $TFIIF_\alpha$, enhances transcription by only about 3-fold. In contrast, when TBP is replaced by TFIID, transcription becomes strictly dependent on TFIIE. Formation of the complete initiation complex still requires the recruitment of TFIIH, a multisubunit complex with CTD kinase (36), ATPase, and helicase activities, which is also involved in nucleotide excision repair of DNA (9, 37). While the minimal complex is sufficient for initiation, TFIIE, TFIIH, and ATP are required for efficient transcription. We will summarize the function of these factors when discussing promoter clearance and efficient elongation (see below) (38). In addition, TFIIA and other factors contribute to basal transcription of certain promoters and participate in interactions with transactivators.

TFIIA, although not essential under *in vitro* conditions, helps to stabilize the binding of TFIID to DNA by eliminating an otherwise inhibitory effect of the nonconserved N terminus of TBP. TFIIA decreases the effect of TATA box- flanking sequences on TFIID binding, suggesting that TFIIA allows TFIID to recognize a wider range of promoters (39). TFIIA purified from HeLa extracts consists of three subunits of molecular masses 35, 19, and 12 kDa, all of which have been cloned (40–42). Recombinant TFIIA with a polypeptide composition similar to that of natural TFIIA supports the formation of a DNA-TBP-TFIIA complex and mediates both basal and Gal4-VP16-activated transcription by RNAP-II in TFIIA-depleted nuclear extracts. The p55 (the precursor of p35 and p19) and p12 recombinant subunits of TFIIA interact with TBP (43). Substitutions of the lysine residues in the basic domain on the convex surface of TBP lead to conditional mutants defective for interaction with TFIIA *in vitro* (44).

Binding of TFIIA to the TFIID-promoter complex is a rate-limiting step that is enhanced by some transactivators. The activation domains of the viral transactivators Zta and VP16 bind to TFIIA, and the ability of mutants to bind TFIIA correlates with their transactivation potential (45). These transactivators induce the formation of a TFIID-TFIIA complex on DNA, which acquires the ability to bind TFIIB in a TFIIA-regulated fashion (46). There are also activators in the HeLa cell extracts that interact with TFIID and act by enhancing the formation of TFIID-TFIIA-containing initiation complexes (47).

The HeLa nuclear extract contains transcriptional suppressors that interact with TFIID through sequences similar to the TFIIA core region (48) and copurify with the TFIIA fraction (49). Some of these suppressors seem to act by binding to TBP and preventing the interaction with TFIIA (50) or with the TATA box region (51, 52). The activity of one of these suppressors, Dr1 (also called NC2), is controlled by phosphorylation. Phosphorylated Dr1 can associate stably with TBP on a TATA box and displaces TFIIA. Dephosphorylated Dr1 cannot associate stably with TBP but can join a TFIIA-TBP-TATA box complex and precludes the association of TFIIB with the preinitiation complex (50). Another factor, Dr2 (also called PC3), represses basal transcription, and this effect can be overcome by TFIIA or transactivators (53). The effect of Dr2 is specific for TATA box-containing promoters and is mediated by TBP. Dr2 is human topoisomerase I but, intriguingly, its relaxation activity is dispensable for transcriptional repression.

In conclusion, the role of TFIIA appears to be less essential for basal transcription and more important for activated transcription. Its activity depends on the specific organization of the core promoter and on interactions with other less characterized factors.

1. TBP-associated factors (TAF_{II}s). Whereas the TBP-based minimal transcription system is unable to respond to activation by sequence-specific transactivators, systems containing TBP complexed to TAF_{II}s in TFIID acquire this property and can respond to such factors (54, 55). The TAFs in TBP complexes used in transcription by the three eukaryotic

TABLE 2. Comparison of human, *Drosophila*, and yeast TAF_{II}s

Human		*Drosophila*		Yeast	Interactions	Homologies
230/250[a]	------	250[a]	------	145/130[a]	d110, h80/70, h55, TBP, TFIIFα, E1A, Rb, DNA	CCG1
?		150[a]	------	150[a]	NTF1, DNA	TSM1
135		110[a]			d250, SP1, CREB; ATF2, Bicoid, SV40Tag, YY1, PR, RXR, THR	
115						
100		80[a]	------	90[a]		Transducin repeats
95[a]						
80/70[a]	------	60[a]	------	60[a]	h250, d40, h31, h20, Hunchback	Histone H4
55[a]					TBP, TFIIEα, TFIIFα, p53, h250, SP1, YY1, USF, CTF, E1A, HIV-Tat	
31/32[a]	------	40[a]		40	h80/70, p53, VP16, TFIIB, DNA	Histone H3
30/31	------	30β[a]		30	d250, d150, h28, h18, ER, SUG1	
28						
20	------	30α[a]		25	d250, d110, h30, h18, TBP	Histone H2B
		22[a]			d250, d110, h80/70, TBP	
19		21				
18					h30, h28	

[a] Cloned TAFs; ------, homologous TAFs.

RNAPs are different. The RNAP-I transcription factor SL1 contains three TAF_{II}s, with molecular masses of approximately 110, 63, and 48 kDa (56), which are distinct from the TAF_{II}s identified in TFIID (molecular mass indicated in Table 2) and from those found in TFIIIB (molecular mass of 190, 96, 87, and 60 kDa) (57). It appears that no common subunit is shared by these three different TBP complexes.

TFIID is present in HeLa cell extracts in several forms with different polypeptide compositions (58, 59), but in *Drosophila* nine different polypeptides are accepted as bona fide TBP-associated factors or TAF_{II}s (55, 60). The existence of a TFIID complex in *S. cerevisiae*, which was questioned, has been confirmed recently (61, 62). TAF_{II}s are structurally and functionally conserved between yeast, *Drosophila*, and human (Table 2), and they assemble into the TFIID complex by contacting analogous subunits. Although the activity of TBP mutants *in vivo* does not correlate with DNA binding or basal transcription *in vitro*, it does correlate with binding to the largest subunit of TFIID, $hTAF_{II}250$ (63).

$TAF_{II}250$ acts as a molecular scaffold in the assembly of other TAF_{II}s with TBP into a functional TFIID complex. $TAF_{II}250$ interacts with the large subunit of TFIIF, TFIIFα or RAP74, and this may contribute to the assembly of the active transcription complex (64). TBP and $TAF_{II}250$ are sufficient for basal transcription. Activation by nuclear factor-1 (NF-1) requires the addition of $dTAF_{II}150$, whereas activation by SP1 needs the addition of $dTAF_{II}110$ (65). $dTAF_{II}110$ contains a glutamine-rich activation domain similar to that of SP1 and can interact with SP1, cAMP response element-binding protein (CREB), activation transcription factor-2 (ATF-2), SV40 large T antigen, and YY1, but not with other transcription factors, such as NF-1, or with TBP (66). On the other hand, $TAF_{II}150$ enhances Sp1 transactivation without direct protein interaction (67).

Binding of TAF_{II}s to core elements directs promoter selectivity by RNAP-II. UV cross-linking of TFIID to the heat shock protein (hsp)70 TATA box region yields radioactive proteins of 250 and 150 kDa corresponding to $TAF_{II}250$ and $TAF_{II}150$, suggesting that both TAF_{II}s directly contact DNA. TBP, $TAF_{II}250$, and $TAF_{II}150$ are minimally required for efficient utilization of the initiator and downstream promoter elements. Depending on the promoter structure, TAF_{II}s can increase (in templates with downstream elements) or decrease (in templates without downstream elements) the stability of TFIID-promoter interactions. The sequences downstream of position −3 are also essential for TAF requirements (68).

$dTAF_{II}80$ contains seven tandem WD40 or transducin repeats present in a family of proteins with diverse biological roles, including signal transduction, cell-cycle regulation, splicing, and transcriptional repression. It has been postulated that $dTAF_{II}80$ could be involved in repression. $dTAF_{II}80$ is unable to bind TBP directly and does not interact strongly with $dTAF_{II}250$, but it is able to interact with a complex containing TBP, $dTAF_{II}250$, $dTAF_{II}110$, and $dTAF_{II}60$. This suggests that formation of TFIID may require an ordered assembly of TAF_{II}s, some of which bind directly to TBP while others are tethered to the complex as a result of specific TAF/TAF interactions.

The activation domain of p53 binds $dTAF_{II}60$ (homologous to $hTAF_{II}80/70$ and to $yTAF_{II}60$) and $dTAF_{II}40$ (homologous to $hTAF_{II}31$). A double-point mutant of p53, which inhibits transcriptional activation, blocks binding to both TAF_{II}s. A partial TFIID complex containing TBP, $TAF_{II}250$, $TAF_{II}60$, and $TAF_{II}40$ supports transactivation by GAL4-p53 (69). *Drosophila* $TAF_{II}60$ and its human homolog interact with $TAF_{II}250$ and TBP, through two C-terminal regions, and with human $TAF_{II}31$ and $TAF_{II}20$, through their N-terminal 100 amino acids. It also interacts with GTFs such as TFIIEα and TFIIFα. The interactions between $hTAF_{II}80/70$ and GTFs could be important during transactivation. A conformational change involving $hTAF_{II}80/70$ could reverse a possible exclusion by TBP and $TAF_{II}250$ (or by other TAF_{II}s) of TFIIE and TFIIF interactions with TFIID.

TBP, $dTAF_{II}60$, and $dTAF_{II}250$ form a stable ternary complex. Both $dTAF_{II}60$ and $hTAF_{II}80/70$ also interact with $dTAF_{II}40$ (70), suggesting they serve an important function in mediating assembly of other TAF_{II}s. $dTAF_{II}40$ is the homolog of $hTAF_{II}31/32$. It has some limited similarity to histone H3 over a short region in the N-terminal half and interacts with the activation domain of p53 and VP16, as well as with TFIIB, but does not bind directly to TBP. Thus, several sets of interactions, including TAF-TAF, TAF-GTF, and TAF-transactivator contacts, are conserved between *Drosophila*

and humans. A complex containing TBP, $hTAF_{II}250$, and $hTAF_{II}70$ does not appear to be sufficient for eliciting efficient activation, but inclusion of $hTAF_{II}31/32$ renders the complex capable of mediating transcriptional activation by GAL4-VP16. Perhaps $hTAF_{II}31/32$ and $hTAF_{II}70/80$ act cooperatively as coactivators for VP16 or, alternatively, $hTAF_{II}31/32$ may stabilize the $hTAF_{II}70/80$-$hTAF_{II}250$ interaction in the partial complex. Since $hTAF_{II}70/80$, $hTAF_{II}31/32$, and $hTAF_{II}20$ show sequence similarities to the protein fold of histones H4, H3, and H2B, respectively, they may form a core structure within the TFIID complex (71).

Human $TAF_{II}55$ (to which there is no known homolog in *Drosophila*) interacts with $TAF_{II}250$ and with multiple transactivators, including Sp1, YY1, USF, HNF-1, E1A, and HIV-Tat. Intriguingly, the interacting region on Sp1 was localized to its DNA-binding zinc finger domain and not to the glutamine-rich transactivation domain (72). The smaller TAF_{II}s have been recently cloned (73, 74) and one of them, $hTAF_{II}30$, appears to play a role in transactivation by estrogen receptor (see below).

TAF_{II}s have also been shown to be essential for a key feature of eukaryotic transcription, namely the synergism between different transactivators. The complex of TBP, $dTAF_{II}250$, $dTAF_{II}110$, and $dTAF_{II}60$ mediates synergism between *bicoid* and *hunchback*, two *Drosophila* transactivators that normally synergize *in vivo* (75). This is achieved by a multivalent interaction of each of the transactivators with a particular TAF, leading to synergistic recruitment of TFIID to the promoter (75).

2. *Promoter clearance and control of polymerase pausing.* TBP, TFIIB, TFIIF, and the nonphosphorylated form of RNAP-II, RNAP-IIA, are sufficient for the formation of functional initiation complexes on both linear and supercoiled templates, but TFIIE and TFIIH, as well as ATP hydrolysis, are required for promoter clearance on linear templates (38). Promoter clearance is a term describing the conversion of an initiation complex into an elongating complex and is probably accompanied by phosphorylation of the CTD region of RNAP-II, likely leading to a dissociation from TBP. TBP binds only to the nonphosphorylated form of RNAP-II, whereas the form of RNAP-II involved in transcription elongation, RNAP-II0, is phosphorylated at the CTD. This suggests that phosphorylation serves to release or disengage RNAP-II from the promoter, at least in part by disruption of the interaction with TBP. A phosphatase that specifically dephosphorylates the CTD of RNAP-II has been characterized (76).

TFIIE, a heterotetramer containing two subunits of relative molecular mass 57 kDa ($TFIIE_{\alpha}$) and two of 34 kDa ($TFIIE_{\beta}$) (77), binds selectively to the nonphosphorylated form of RNAP-II, and this interaction is mediated by the 57-kDa subunit of TFIIE (20). Additionally, $TFIIE_{\alpha}$ can interact with TBP as well as TFIID. TFIIE also interacts with both subunits of TFIIF and with TFIIH, a multisubunit basal factor reported to catalyze phosphorylation of the CTD of RNAP-II (36). Protein affinity assays demonstrate that TFIIE binds directly to ERCC-3, a DNA repair protein associated with TFIIH. More importantly, a TFIIE affinity resin can selectively isolate transcriptionally competent TFIIH from a partially purified preparation, suggesting that TFIIE may recruit TFIIH to the transcription complex *in vivo* (78). The N-terminal half of TFIIE is sufficient for CTD binding and the C terminus is required for TFIIH recruitment (79). These multiple interactions between TFIIE, RNAP-II, and TFIIH support a model in which TFIIE plays a role in promoter clearance as well as in the recruitment of proteins required for transcription-coupled DNA repair (78).

Transcriptionally active yeast TFIIH consists of three components: a five-subunit core, the SSL2 product, and a complex of three polypeptides (47, 45, and 33 kDa) that possesses CTD kinase activity (80). This kinase complex is essential for transcription. By contrast, TFIIH active in nucleotide excision-repair (NER) lacks the kinase complex and contains all the other NER-relevant gene products: RAD1, 2, 4, 10, and 14. This repairosome is not active in transcription (80). Cdk-activating kinase [CAK, composed of human cyclin H (homologous to yeast CCL1), Cdk7/MO15 (homologous to yeast KIN28), and MAT1] is a component of human transcription factor TFIIH. Antibodies to cyclin H inhibit TFIIH, whereas antibodies to cyclin H or to Cdk7 inhibit CTD phosphorylation. The third component of CAK, MAT1 (menage a trois 1), encodes a new RING finger protein with the C_3HC_4 motif at the N-terminal end, which stabilizes the cyclin H/Cdk7 complex (81). CAK is present in two complexes: TFIIH and a smaller complex unable to phosphorylate CTD in the initiation complex. However, both CAK complexes, as well as recombinant CAK, phosphorylate a CTD peptide. TFIIH also phosphorylates Cdc2 and Cdk2, and this could link transcription to the cell cycle (82). Efficient transcription, probably involving promoter clearance, requires CTD kinase activity provided by the Cdk7 subunit of TFIIH (83).

All the GTFs coexist in mature initiation complexes but, after nucleotide addition, TFIID remains bound to DNA while TFIIB, TFIIE, TFIIF, and TFIIH are released. Upon release, TFIIB reassociates with TFIID, reforming a RNAP-II docking site, the DB complex. TFIIE is released before formation of the tenth phosphodiester bond, preceding TFIIH release, which takes place after 30 phosphodiester bonds. TFIIF is the only basal factor detected in the elongating RNAP-II complex and, upon release, can reassociate with stalled RNAP-II (84). In a highly purified enzyme system that supports CTD phosphorylation and basal transcription, inhibition of CTD kinase does not preclude basal transcription when RNAP-II has already entered the initiation complex but diminishes the rate of formation of the initiation complex (85).

In vitro transcription data suggest that initiation by RNAP-II in the absence of transcriptional activators is limited by melting of the promoter DNA upstream of the initiation site, as required for the formation of an open complex (86). Yeast RNAP-II melts DNA 20 bp downstream of the TATA box, as in animal cells, and the polymerase scans DNA for an initiation site (87). Dependence on TFIIE varies among promoters, increases with salt concentration, and is dictated by the region between −10 and +10 (88). Recent results suggest that the CTD repeat of RNAP-II is also needed for efficient initiation on a TATA-less promoter, whereas its phosphorylation by the kinase activity of TFIIH is important for promoter clearance (83).

The topology of the general TFs (TBP, TFIIA, TFIIB, and

TABLE 3. The mediator and the RNAP-II holoenzyme complex

Name	Structure, homologs	Phenotype features
Mediator		
TFIIF	2 subunits (74 & 30) RAP74, RAP30	Binds Pol-II, $TAF_{II}250$, $TAF_{II}80/70$, elongation
GAL11	SPT13	Initiation, binds activators
SUG1	TRIP1	Interaction with T3R, ER, $TAF_{II}30$
SRB2	23 kDa	Dominant, conditional, binds TBP, suppresses Hpr1
SRB4	78 kDa	Dominant, deletion inviable
SRB5	34 kDa	Dominant, conditional, binds TBP
SRB6	14 kDa	Dominant, deletion inviable
+7 additional unknown proteins		
Holoenzyme contains also:		
RNAP-II	12 subunits	Interacts with basal and regulatory factors
TFIIB		Bridges Pol-II and TBP
TFIIH	5 subunits	DNA dep. ATPase, DNA helicase
		CTD kinase (MO15)
		Promoter, clearance, repair
SRB7	140 aa	Recessive, deletion inviable
SRB8	1226 aa	Recessive, conditional
SRB9	1420 aa	Recessive, conditional
SRB10	61 kDa	Recessive, conditional, kinase acting on CTD
SRB11	38 kDa	Recessive, conditional cyclin-like linked to SRB10

aa, Amino acids.

TFIIFβ) along the adenovirus major late promoter has been studied by site-specific tagging with the photoactivable cross-linking reagent N3R-dUMP (89). TFIIA cross-links to the coding strand opposite to TBP at the TATA box and upstream of TBP around position −40. TFIIFβ cross-links strongly and TFIIB cross-links weakly to the coding strand just downstream of TBP at −19. During transcription of a supercoiled template, the RNAP remains at the top of a moving loop, thus precluding circling of the enzyme around the DNA template and entangling of the nascent RNA transcript (90).

After formation of an open complex and synthesis of a few nucleotides, additional controls regulate the rate of effective polymerase elongation. For instance, the c-*fos* promoter is not only controlled by factors binding upstream of the initiation site, but also by an intragenic regulatory element located at the end of the first exon. This site controls transcriptional elongation and acts in conjunction with the serum response element, which controls initiation (91). *In vitro* transcription experiments identified a unique arrest site within the first intron, 385 nucleotides downstream from the promoter. A 103-nucleotide long motif comprising the arrest site is sufficient for obtaining the elongation block in a cell-free transcription assay (92). High resolution run-on analysis and *in vivo* $KMnO_4$-dependent footprinting identify engaged RNAP-II molecules on the c-*fos* gene in unstimulated cells at the place where the block to transcription elongation takes place (93). A similar situation may apply to the c-*myc* gene, in which RNAP-II is held at the P2 promoter (94, 95).

Probably the best characterized example of controlled transcription elongation is found in the *hsp70* gene of *Drosophila*. The number of RNAP-II molecules associated with several heat shock genes, as measured with a cross-linking method, increases dramatically in response to heat shock (96). In the *hsp70* gene of induced cells, a high density of RNAP-II molecules is detected on the entire gene, while in noninduced cells, the RNAP-II is confined to the 5'-end, predominantly between nucleotides −12 and +65 relative to the start of transcription (97). Before the induction, there is approximately one molecule of RNAP-II associated with the hsp70 promoter region. This polymerase is transcriptionally engaged and has formed a nascent RNA chain of approximately 25 nucleotides in length but is apparently arrested and unable to penetrate further into the hsp70 gene without heat induction (98). The detection of a postinitiation RNAP complex on the promoter region of the inactive gene suggests that there is a transcriptional control mechanism that acts at an early step in transcript elongation.

There are sequence similarities in the promoters of genes regulated by elongation control, such as c-*myc*, *Drosophila hsp70*, and human immunodeficiency virus-1, suggesting that a conserved mechanism may be operative. The high transcription of the promoter-proximal region, with the production of short transcripts, may provide a general mechanism for preventing repression by chromatin (99) or for creating a chromatin structure competent for binding of transactivators (100).

The importance of transcription elongation in the control of gene expression and cell proliferation is underlined by the recent demonstration that the tumor suppressor protein VHL, which is mutated in families with von Hippel-Lindau disease, functions as an inhibitor of transcriptional elongation. It acts by virtue of its interaction with the heterotrimeric elongin complex, which activates transcription elongation *in vitro* (101–103). Association of the VHL protein with two subunits of the complex prevents binding of the active elongin subunit and inhibits the activity of the complex.

C. Holoenzyme recruitment

An alternative to the sequential recruitment of individual GTFs is the existence of preformed complexes, including the RNAP-II and many general GTFs, that could be directly recruited to the promoter by sequence-specific transcription factors (Fig. 1). Such a holoenzyme containing RNAP-II, TFIIB, TFIIF, TFIIH, suppressor of RNA polymerase B (SRB)

gene products, and several additional polypeptides (Table 3), exists in yeast (104). A subcomplex called "mediator" and including TFIIF, GAL11, SUG1, SRB2, SRB4, SRB5, SRB6, and several other polypeptides mediates transactivation by GCN4 or GAL4-VP16 in a cell-free system based on RNAP-II and GTFs (105). The response of the holoenzyme to transactivators takes place with TBP and does not require TAF_{II}s, suggesting an alternative pathway for transactivation.

The SRB genes were isolated as suppressors of mutations in the CTD region of the largest subunit of RNAP-II (106), which is not required for basal transcription but is needed for transactivation by some enhancers or for signals from the upstream activation sequence (107). Two of them, SRBs 10 and 11, encode kinase- and cyclin-like proteins, respectively, which form a pair in the holoenzyme and are essential for Gal induction *in vivo* (108). Holoenzymes lacking SRB10/11 are deficient in CTD phosphorylation but function normally in defined *in vitro* transcription systems with naked DNA templates. Similar activities have been found in mammalian TFIIH (109).

SRB proteins may play a key role in activated transcription, as anti-SRB antibodies precipitate VP16, and a VP16 column binds holoenzyme together with TBP, as well as mediator (110). Another component of the holoenzyme, SUG1, is a member of a highly conserved family of ATPases, which interacts with the acidic domain of the transactivators Gal4 and VP16 and nuclear receptors, as well as with TBP (111).

That recruitment of the holoenzyme may be essential for activated transcription is suggested by the behavior of the GAL11p mutation. This mutation confers to GAL11, a component of the holoenzyme, the ability to interact with the dimerization domain of GAL4 and leads to transactivation by GAL4 mutants lacking the transactivation domain (112). Using temperature-sensitive mutations in SRB genes, one can calculate the fraction of genes that require SRB proteins *in vivo* for transcription by RNAP-II. Upon transfer to the restrictive temperature, there is a rapid and general shutdown of mRNA synthesis in *srb* mutant cells. As essentially all of the SRB protein fraction in cells is tightly associated with RNAP-II molecules, it seems that SRB-containing holoenzymes are the form of RNAP-II recruited to most promoters in the cell (113). Very recently, components of the cellular machinery involved in chromatin remodeling and modification of core histones have been identified as potential components of the holoenzyme complex (see below).

A mammalian RNAP-II holoenzyme has been recently isolated using a monoclonal antibody to MO15/CDK7 to precipitate nucleoproteins from rat liver nuclear extracts (114). This holoenzyme contains RNAP-II, TFIID, TFIIB, TFIIH, TFIIF, and TFIIE but no transactivators. It can initiate transcription *in vitro* without further additions but, surprisingly, does not respond to sequence-specific transactivators *in vitro*.

III. Interaction of Steroid Hormone Receptors with Transcription Factors

We will divide the discussion of this issue into two main sections, one dealing with direct interactions with the GTFs and the other dealing with the recently discovered class of transcription-intermediary factors, which mediate interactions of receptors with GTFs.

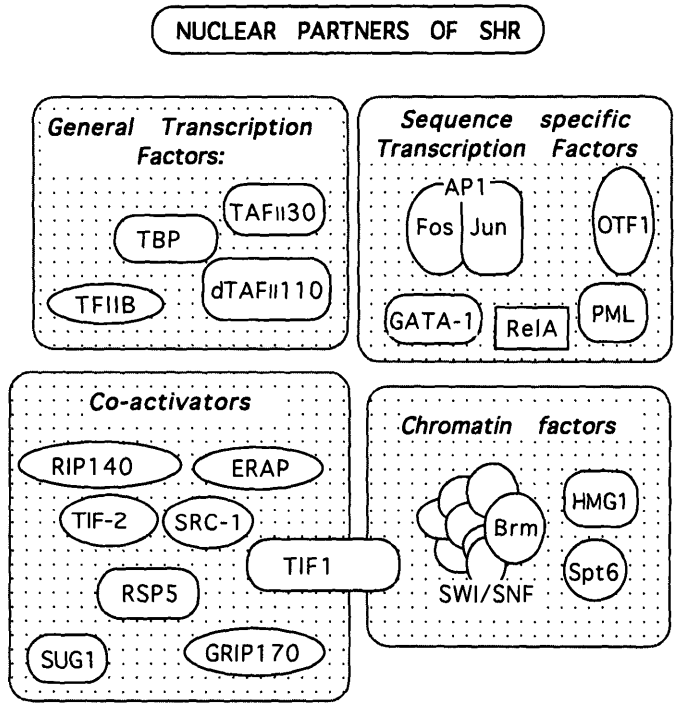

FIG. 2. Nuclear partners of steroid hormone receptors. Nuclear proteins which have been shown to interact with steroid hormone receptors are grouped in four classes: GTFs, sequence-specific transcription factors, coactivators, and chromatin-related factors. The coactivators TIF1 is shown bridging to the chromatin factors, as it has been shown to interact with chromatin proteins (B. LeDouarin, P. Chambon & R. Losson, unpublished). For further details and references see Tables 1, 2, and 3, and the text.

A. Direct interactions with GTFs

As already mentioned, many sequence-specific transactivators interact directly with various components of the general transcription machinery. The best characterized transactivator is perhaps the viral protein VP16. Its acidic activator domain interacts with TBP, with TFIIB, and with several TAF_{II}s (Table 2). The binding of VP16 to TFIIB disrupts an intramolecular interaction of the N- and C- terminal regions of TFIIB and exposes interfaces for interaction with other GTFs (115). In this way it may help to recruit RNAP-II and other factors.

Steroid hormone receptors seem to be able to interact with several components of the general transcriptional machinery (Fig. 2). In transfections and in cell-free transcription assays, steroid hormone receptors can activate minimal promoters (116, 117). Estrogen receptor (ER) transactivation is enhanced in response to overexpression of TBP, and the two proteins interact *in vitro* (118). This interaction, however, can not be sufficient for transactivation or must be modulated by TAFs, since the receptors cannot activate transcription of the rRNA genes by RNAP-I, which is also catalyzed by a TBP-containing complex (119). TAF_{II}s have been identified as potential targets of hormone receptors. Human $TAF_{II}30$ is required for

TABLE 4. Transcription intermediary factors for steroid hormone receptors

TIF	Class	Receptor		Target	Reference
		Partner	Domain		
ERAP		ER	?	?	(139)
RIP140		ER, GR	AF2	?	(141)
GRIP170		GR/GRE	DBD	?	(142)
RAP46		GR	?	?	(144)
SPT6		ER	LBD	?	(143)
TIF1	RING	RXRγ, ER, GR,...	AF2	?	(145)
PML	RING	PR	AF1, AF2		(146)
SUG1	Holoenzyme	T_3R, RAR	AF2	TBP	(148)
				$TAF_{II}30$	(149)
SRC-1		PR, ER, GR,...			(150)
RSP5	Ubiquitin ligase	GR, PR		$TAF_{II}18$?	(151)

transactivation by ER and both proteins interact *in vitro* (120). *Drosophila* $TAF_{II}110$ interacts with the DNA- binding domain of progesterone receptor (PR) (121) but also with retinoic acid receptor RXR and with the thyroid hormone receptor (122). However, the specificity of these interactions seems to be limited since $dTAF_{II}110$ has also been shown to mediate transactivation by Sp1 (66), CREB (123), and other transactivators (see Table 2). An interaction with TFIIB has been well documented for thyroid hormone receptor (124, 125) and for the vitamin D_3 receptor (126, 127). A similar interaction between ER or PR and TFIIB has also been reported (128), but its significance remains to be established (121).

B. Indirect interactions mediated by transcription-intermediary factors (TIFs)

Coactivators or transcriptional adaptors, which are required for transactivation by viral (129) and cellular factors (130–135), have been described. They are supposed to bridge between sequence-specific transactivators and the GTFs. For instance, CBP binds selectively to the phosphorylated form of CREB and can activate transcription through a C-terminal region that interacts with TFIIB (131). The suspicion that steroid hormone receptors may require coactivators was derived from the "squelching" phenomenon (136). Squelching refers to the fact that excess receptor can inhibit its own transactivation function as well as transactivation by other transactivators (137–140).

Recently, a number of potential TIFs for steroid hormone receptors have been identified (Table 4 and Fig. 2). Several proteins interact with the ligand-binding domain of ER in a ligand-dependent manner (141, 142). One of them, RIP140, which has been cloned, is a 1158-amino acid protein with two charged domains and a Ser/Thr cluster, which interacts with ER through the C-terminal half. Transcriptionally inactive mutants of ER carrying amino acid exchanges at the activation function AF2 do not bind RIP140. Evidence for an interaction with ER in the intact cell is derived from colocalization and transfection studies in which a weak potentiation of transactivation by ER is observed at low levels of RIP140 (143).

Using a biochemical strategy, a ubiquitous protein, called GRIP170, has been isolated, which binds to a complex of glucocorticoid receptor (GR) and two glucocorticoid-response elements and enhances activation of transcription by GR in a cell-free system without influencing basal transcription (144). A protein with similar properties, but which interacts with the DNA-binding domain of ER, has been identified in yeast (145). Screening of a λgt11 expression library with GR led to the identification of a 46- kDa protein that binds to the activated forms of GR and ER independent of ligand (146).

Using a yeast two-hybrid screen with RXRγ, a protein interacting with its conserved activation function AF2, which also interacts with ER and PR in a ligand-dependent way, has been identified and cloned (147). However, this protein, called TIF1, does not interact with components of the general transcriptional machinery and interferes with RXR transactivation in transfection assays. Interestingly, TIF1 belongs to a group of RING proteins with two B boxes, which also includes PML. PML, the transcription factor to which RARα is fused in acute promyelocytic leukemia, has been independently shown to enhance transactivation by PR, without influencing its ligand binding or its affinity for DNA (148). Another member of the same family of proteins is *efp*, which was cloned as an estrogen responsive gene (149). If *efp* could be shown to potentiate hormone receptor transactivation, a cascade of hormone regulation could be postulated.

Another interesting auxiliary factor, namely SUG1 (also called Trip1), was identified in yeast genetic screens with thyroid hormone receptor (150) and RAR (151). Since SUG1 is a component of the RNAP-II holoenzyme, it could be used by nuclear receptors to recruit the complete transcriptional machinery in a single step. In fact, SUG1 also interacts with the transactivation domains of GAL4 and VP16, as well as with TBP (111) and $TAF_{II}30$ (151), and, therefore, fulfils the requirement for a bona fide TIF. Although SUG1 and TIF1 both interact with the AF2 of nuclear receptors, the details of the interaction are different and the relative affinities of the two proteins for various members of the superfamily are also different (151). Recently SUG1 has been shown to be part of the 26S proteasome, suggesting that the effect of SUG1 mutations on transcription may be indirect and result from defective proteolysis. This finding also underlines the dangers of the two-hybrid screen strategy and the necessity of confirming its results with other functional assays.

SRC-1, a ubiquitous factor isolated by the two-hybrid system in yeast, interacts with PR *in vitro* in a ligand-dependent fashion and enhances transactivation by PR, ER, GR, TR,

RXR, VP16, and Sp1 (but not CREB or E2F). An N-terminal truncated SRC-1 acts as a dominant negative repressor of steroid hormone (152). RSP5, also detected in a two-hybrid screen in yeast, and a human homolog, hRPF1, potentiate GR and PR transactivation in animal cells (153). RSP5 could be a ubiquitin-protein ligase and acts in yeast in conjunction with Spt3, which contacts TFIID. Spt3 is likely the yeast homolog of $hTAF_{II}18$, which forms the TFIIDβ complex also containing hTAFII28 and hTAFII30 and known to mediate ER transactivation (120).

In the case of thyroid hormone receptors and RAR, the action of positive coactivators presupposses the ligand-dependent displacement of negative receptor-interacting proteins or corepressors (154–156). The two corepressors identified so far (N-CoR and SMRT) do not interact with GR or ER, but since they seem to be part of a gene family, the possibility remains that other members of the family target steroid receptors. Additional proteins have been identified in genetic screens for intermediary factors (157), and many more can be expected to be found in the near future. These findings underline the complexity of the molecular mechanisms involved in transactivation by hormone receptors. One of the open challenges in the field is the identification of the physiological function of these receptor-interacting proteins, which will require manipulating their normal concentration in the target cells by antisense or gene-targeting techniques.

C. Interactions with sequence-specific transcription factors

Normally, steroid hormone receptors control the activity of natural promoters not only by contacting GTFs directly or indirectly but also through positive and negative interactions with sequence-specific transcription factors (Fig. 2). Under physiological conditions this kind of interaction, which can result in functional synergism or repression, plays an essential role in modulation of promoter efficiency. However, a detailed description of the known interactions is beyond the scope of this review, which is focused on the effects of receptors on the transcription initiation complex. Inasmuch as the interaction with sequence-specific factors ultimately determines the rate of initiation, we will very briefly summarize them here (for a more extensive review on this topic see Refs. 158 and 159).

In artificial promoters, HREs can synergize with binding sites for a variety of transcription factors, provided they are located at an appropriate distance (160). On natural promoters the situation is more complex. For instance, on the MMTV promoter, CTF1/NF-1, as well as Oct1/OTF1, has been shown to be required for optimal induction by either glucocorticoids or progestins. On naked DNA templates the receptors do not synergize but rather compete for binding and activation of the MMTV promoter (161, 162), and there are indications that chromatin organization of the promoter is essential for this synergism (see below). A direct interaction between GR and PR with Oct1/OTF1 has been described (163), but the functional synergism observed *in vivo* is probably also mediated by the organization of the relevant DNA sequences in chromatin (see below). A similar situation may apply for the interactions between GR and HNF-3 on the enhancer of the rat tyrosine aminotransferase gene (164).

Steroid hormone receptors can also interact directly with sequence-specific factors in a way that mutually represses the activity of both partners. The classic example for this type of interaction takes place with the heterodimeric transcription factor AP1 (for a recent review see Ref. 165). Typically this mutually inhibitory interaction may take place on composite sites (containing HREs and AP1 sites) or on separate HREs or AP1 sites, and the outcome of the interaction depends on the composition of the AP1 dimer. Similar interactions have been recently described between GR and the RelA subunit of the transcription factor NF-κB (166–170). These interactions may be very important for the antiinflammatory and immunosuppressive effects of glucocorticoids, although an indirect effect through induction of the NF-κB inhibitor I-κB may also contribute (171, 172). Finally, a direct negative interaction between GR and the transcription factor GATA-1 has also been reported (173).

IV. Modulation of Receptor Interactions

The interactions of steroid hormone receptors with the factors described above, as well as with DNA, are modulated by a number of parameters, such as the state of phosphorylation of the receptors and of the factors, which vary in response to activation of other signal transduction pathways. We will not discuss these aspects here but will concentrate on the role of the ligand and, in particular, on the influence of chromatin organization.

A. Role of the hormone ligand

A classic principle of endocrinology is that the function of the hormone receptors is activated by binding of the agonistic ligand, the hormone. However, in the case of the steroid hormone receptors, the elucidation of the molecular mechanism of the ligand action remains elusive. This is important in view of the need for antagonistic ligands that permit a pharmacological control of receptor function for the treatment of endocrine tumors and other disorders. In the original concept of receptor function, the ligand was supposed to activate the receptor in terms of nuclear translocation, DNA binding, and gene activation (174). The recent elucidation of the structure of the liganded retinoic acid receptor-γ suggests a molecular basis for the ligand effect on transactivation, as ligand binding induces a conformational change of the C-terminal activation region AF2, which exposes a amphipathic α-helix for interaction with TIFs (175). However, there is no consensus in how hormone binding influences the various steps in receptor action and on whether hormone binding is the only way of activating receptors. Some receptors are mainly nuclear in the absence of hormones, and it has been claimed that the receptors can recognize their DNA target sequences in the absence of hormone or when complexed with antagonistic ligand. Part of the confusion may be explained by the recent demonstration that steroid receptors can be activated in the absence of the cognate hormone through signals originating in membrane receptors (for a recent review of this issue, see Ref. 176 and references therein). These other signal transduction pathways can modulate the activity of the hormone-receptor

complex by mechanisms probably involving the control of phosphorylation events. The idea is gaining momentum that the steroid hormone receptors act as signal integrators, sensing a variety of other signaling systems, and at the same time influencing the function of these other pathways. Through this intensive cross-talk, the cell coordinates different signals from the microenvironment into a balanced and physiologically meaningful metabolic response. This aspect will not be further discussed here. It may suffice to say that this cross-talk implies a modulation of the interaction of the hormone receptors with general and sequence-specific transcription factors described above, not only by virtue of ligand binding, but also by alternative means.

B. Role of chromatin

Eukaryotic DNA is organized in chromatin within the cell nucleus. The basic unit of chromatin is the nucleosome, a flattened disk 11 nm in diameter and 5.7 nm in height. At its center, there is a wedge-shaped protein octamer with two each of the histones H3, H4, H2A, and H2B, around which are wound 145 bp of DNA forming 1.8 negative superhelical turns. The histone octamer is composed of a central $(H3\text{-}H4)_2$ tetramer flanked by two H2A/H2B dimers and is assembled to form a left-handed protein superhelix (177). On the surface of this structure, there is a distribution of positively charged residues that fits the path of the DNA, in such a way that the central 12 turns of the DNA contact the octamer at repetitive structural motifs (177). The nucleosomal fiber is folded into less well defined higher order structures, partly by the cooperative binding of linker histones (178).

A participation of chromatin organization in the regulation of gene expression was postulated years ago, but this problem has been approached only recently with appropriate experimental strategies. Two kinds of experimental results have contributed to a revival of this old question: evidence suggesting a participation of the structural organization of DNA by transcription factors as an essential aspect of transcription; and genetic evidence supporting the importance of chromatin in the process of gene regulation. We will revise these two aspects before presenting evidence that the nucleosomal organization of a hormonally regulated promoter may be essential for synergistic interaction between hormone receptors and other transcription factors.

1. Architectural role of transcription factors and nucleosomes. As mentioned above, the most abundant GTF, namely TBP, causes dramatic DNA bending (25), and this may be one of its essential properties, since it facilitates binding of TFIIB to DNA (16) and subsequent recruitment of RNAP-II and TFIIF (24). The first example of a purely architectural role for a transcription factor derives from the discovery that the upstream binding factor on the ribosomal RNA promoter, UBF, contains three regions of homology to HMG proteins (179). When the multiple HMG motifs of UBF bind to DNA they form a disk-like structure that wraps 180 bp of DNA in a right-handed direction and introduces positive supercoiling (180, 181). A similar situation, although with higher structural specificity, is found in the interferon-β (INFβ) enhancer, in which binding of HMG-I(Y) is required for the assembly of transcription complexes containing ATF-2, Jun, and NF-κB, bound to two separate regions of the enhancer (182). Binding of a HMG1-like protein to a negative element can convert members of the Rel family (*Dorsal* and NF-κB), binding to adjacent sites, from activators to repressors (183). Similarly, binding of the HMG domain of LEF-1 to the minor groove bends DNA and enables interactions among other TFs bound to the T cell receptor enhancer (184, 185). Another nonhistone chromosomal protein, HMG17, stimulates chromatin transcription in conjunction with GAL4-VP16, acting at the level of initiation (186). In a reconstituted transcription system, basal promoters can be activated or repressed by HMG-2, a nonhistone chromosomal protein that also induces DNA bending. The inhibitory effect can be counteracted by an ATP-dependent activity in TFIIH (187). The activation requires TFIIA, depends on TAF_{II}s in TFIID, and generates a initiation complex from which TFIIB dissociates more slowly (188). Along these lines, HMG-1 has been reported to stimulate DNA binding of PR and ER, although the physiological relevance of this observation is unproved (189, 190).

Additional support for the relationship between structural components of chromatin and gene regulation comes from the identification of structural homologies between histones and transcription factors. Linker histones contain a structural domain, called the "winged motif," which is also found in the *Drosophila* homeotic gene product fork head (191) and in HNF3, a transcription factor important for the function of the rat albumin enhancer and for the glucocorticoid induction of the rat tyrosine aminotransferase gene (192, 193). Histone H2A and H2B show structural homology to the C and A subunits of NF-Y/CBF, a multimeric transcription factor that, like its yeast homolog HAP, is involved in CAAT box recognition (194). Finally, histone H3 and H4 exhibit structural homology with $TAF_{II}40$ and $TAF_{II}60$, respectively, two TAFs that participate in transactivation by p53 (69).

There are also several examples of a structural role for positioned nucleosomes. In the *Drosophila hsp26* gene, a nucleosome could be involved in bringing together upstream heat shock elements and the TATA box region, thus facilitating interactions among DNA-bound heat shock factor and the general transcriptional machinery (195). A similar situation has been postulated for the vitellogenin B1 gene of *Xenopus*, in which a positioned nucleosome may bring an HRE in apposition to other promoter elements and enable interaction between hormone receptors and other transcription factors (196). An effect of the estrogen receptor on chromatin structure has been reported in yeast strains expressing recombinant receptor (197). Chromatin structure may also facilitate the interaction among distant DNA sequences, as suggested by the "nuclear ligation assay" (198): estrogen treatment stimulates the ligation efficiency 2- to 3-fold between the distal enhancer and the proximal promoter regions of the rat PRL gene, suggesting that these two regions are juxtaposed in minichromosomes after hormonal induction.

2. Genetic evidence for a role of chromatin in transcription. Genetic analysis in yeast has revealed a widespread involvement of chromatin structure in gene regulation. Yeast strains carrying mutated histone genes exhibit multiple alterations of the normal gene expression pattern, including constitutive

TABLE 5. The SWI/SNF complex and its suppressors

Name	Alternative homologs	Phenotype/features
SWI1	ADR6, GAM3	Poly-Asn, poly-Gln, metal ion binding
SWI2/SNF2	TYE3, GAM1/*brahma*	Nuclear ATP-dependent helicase
SWI3		Acidic
SNF5	TYE4	Nuclear, Gln, and Pro-rich
SNF6		Nuclear, acidic region
SWI11		Interacts with SWI2/SNF2
SWI13		Binds cooperatively with GRF10 to HO promoter
Suppressor mutants		
SIN1	SPT2	Nuclear, HMG-1 like
SIN2–3	HHT1	Histone H3
SIN2–4	HHF2	Histone H4
SIN3	UME4, RPD1	175 kDa, PAH. Negative regulator of HO, PHO5, INO1, inhibits PR action in yeast
SPT3	TAFII?	Suppresses pleiotropic TBP mutation, binds TBP, acts synergistically with SPT3 to enhance GR and PR function
SIN4	SSN4/TSF3	Negative regulator of HO, GAL1–10, IME1, positive regulator of MATα2, HIS4
RGR1		Interacts with SIN4
SPT4		Metal ion-binding motif
SPT5		Nuclear, acidic region
SPT6	SSN20, CRE2	Nuclear, acidic region
SPT10		Required for HTA2-HTB2 transcription
SPT11	HTA1	Histone H2A
SPT12	HTB1	Histone H2B
SPT16	CDC68	Acidic
SPT21		Required for HTA2-HTB2 transcription

derepression of inducible genes (199). In the case of steroid hormone receptors, experiments with GR expressed in yeast pointed to a potentially relevant set of receptor-interacting proteins, namely components of the SWI/SNF complex. The SWI/SNF complex includes a set of pleiotropic transactivators that appear to be important for transcription of inducible genes, probably by mechanisms involving remodeling of chromatin (200) (Table 5). A screen for suppressors of the *swi/snf* phenotype identified several mutations in core histones and other structural proteins of chromatin, suggesting that components of the SWI/SNF complex counteract repressing functions of chromatin proteins (200).

Yeast strains with mutations in genes encoding components of the SWI/SNF complex are defective in transactivation by GR, and GR interacts with SWI3 (201). A suppressor of the *SWI/SNF* phenotype, Spt6, potentiates transactivation by ER in yeast and has been postulated to act as coactivator (145) (Table 4). *Brahma* (*brm*), the homolog of SWI2 in *Drosophila*, is a helicase with DNA-dependent ATPase activity (202). The human homolog, *hbrm*, is a 180-kDa nuclear factor that can act as a transcription factor when fused to a heterologous DNA-binding domain. The mouse *brm* is expressed ubiquitously, whereas *hbrm* is not expressed in all cells. In cell lines lacking *hbrm*, such as C33 and SW13, GR induction of a transfected reporter is weak and can be selectively enhanced by cotransfection of a *hbrm* expression vector (203). A second human homolog of yeast SWI2 and *Drosophila brm*, named BRG1, is a nuclear protein of 205 kDa which ATPase domain can, as a chimeric protein, restore GR-dependent transcription in yeast strains lacking SWI2 (204). Like SWI2 in yeast, *hbrm* is part of a large multiprotein complex, which could be involved in the remodeling of chromatin required for transcription and/or act as a bridge between activators and the basal transcriptional machinery. The human SWI/SNF complex isolated from HeLa cells mediates ATP-dependent disruption of a nucleosome containing GAL4-binding sites, and enables binding of transactivators linked to the GAL4 DNA-binding domain to the nucleosome core (205). Moreover, the retinoblastoma gene product (Rb) up-regulates GR-mediated transactivation, which is dependent on *hbrm* (206). Thus, components of the SWI/SNF complex could also act as a connection between gene regulation and the cell cycle.

3. Biochemical evidence for a role of chromatin in transcription. Experiments with reconstituted nucleosomes have provided insight into the role of the primary level of chromatin organization in the control of transcription. A model for rotation of the DNA around a nucleosome that permits a continuous contact with the histone octamer while a prokaryotic RNAP transcribes along the DNA has been proposed (207). In a recent version of this model, RNAP rapidly transcribes the first 25 bp of a short nucleosomal DNA and then continues slowly toward the nucleosome dyad. There, the barrier to polymerase progression disappears and the transcript is completed rapidly, as on free DNA. If DNA behind the polymerase is removed during transcription, the barrier does not appear until the enzyme has penetrated 15 bp farther into the nucleosome (208). The pausing may be due to difficulties in transcribing the DNA in the loop formed when the octamer contacts the DNA behind the polymerase. On a longer template the barrier is almost eliminated, probably because more DNA is available for transient contacts with the histone octamer (208).

Current opinion is that the nucleosomal organization of the templates interferes with transcription initiation but does not hinder elongation (209). However, as available evidence is mostly based on *in vitro* systems and/or on the use of

prokaryotic RNAPs, its physiological relevance is questionable. Neither SP6 polymerase nor mammalian RNAP-II can initiate transcription at a promoter in a nucleosome, but both enzymes can read through and displace nucleosomes (210). However, in other systems, both initiation and elongation are hindered by nucleosomes (211). Although no dissociation of the histone octamer is required for transcription elongation (212), the SWI/SNF complex may facilitate the elongation process (213).

The mode of action of transactivators may, in part, involve counteracting repression due to chromatin organization and, in particular, to linker histones. Reconstitution of nucleosomal cores to an average density of 1 nucleosome per 200 bp of DNA results in a mild reduction of basal RNAP-II transcription to 25 to 50% of that obtained with naked DNA templates. This reduction is caused by nucleosomal cores located at the RNA start site and can not be counteracted by sequence-specific transactivators (214). When histone H1 is incorporated into the chromatin at 0.5 to 1.0 molecule per nucleosome, RNA synthesis is further reduced to 1–4% of that observed with chromatin containing only nucleosomal cores. However, this H1-mediated repression can be counteracted by addition of Sp1 or GAL4-VP16. This phenomenon is called antirepression (214). With naked DNA templates, transcription is increased by a factor of 3 and 8 by Sp1 and GAL4-VP16, respectively (meaning true activation). With H1-repressed chromatin templates, however, the magnitude of transcriptional activation mediated by Sp1 and GAL4-VP16 is 90 and more than 200 times higher, respectively, because of the combined effects of true activation and antirepression (214). The GAL4 DNA-binding domain alone is sufficient to disrupt binding of histone H1 to DNA, but a transactivation domain is needed for antirepression (215). However, the repressive effect of histone H1 may not be observed with regularly organized nucleosomal templates. Transcription of reconstituted chromatin with regularly spaced nucleosomes containing GAL4-VP16, prebound to DNA before reconstitution of either H1-deficient or H1-containing chromatin, yields similar efficiencies (216). Moreover, long distance activation (>1 kb) is found only with H1-containing chromatin and not with naked DNA. Preformed H1-deficient chromatin, but not H1-containing chromatin, can be activated by GAL-VP16 (216). Activation of preassembled chromatin templates containing five GAL4-binding sites is accompanied by chromatin reconfiguration to either side of the GAL4 sites, which requires ATP and an auxiliary activity in the assembly extract (217). This effect requires the transactivation function and is not elicited by a GAL4 DNA-binding domain alone. Probably the transactivators facilitate the assembly of the transcription initiation complex on chromatin templates through their effects on the chromatin organization of the promoter (see below).

4. *The mouse mammary tumor virus (MMTV) promoter: a dual role of nucleosomes.* Indirect evidence supporting a role of the basic structural unit of chromatin, the nucleosome, in gene regulation has also been obtained from studies of regulated systems, such as the MMTV promoter (158). The MMTV promoter is transcriptionally controlled by steroid hormones, in particular glucocorticoids and progestins. The hormone receptors bind to a hormone-responsive region (HRR) and facilitate the interaction of other transcription factors, including NF-1/CTF1 and OTF1/Oct-1 (Fig. 3), with the MMTV promoter (for a review see Ref. 218). A role for chromatin structure in MMTV regulation has been postulated based on functional studies with cell lines carrying stable minichromosomes (219, 220) and on *in vitro* nucleosome reconstitution studies (221–223). The combined results from these two lines of research suggested that while hormone receptors recognize the HRR of MMTV in chromatin, access of the transcription factor NF-1 to the MMTV promoter is hindered by its nucleosomal organization (Fig. 3B). Although the exact positioning of the nucleosome over the MMTV promoter has been debated (224, 225), a dominant nucleosome phase was found that precludes binding of NF-1 (223, 226). A similar situation is found in yeast strains carrying an integrated copy of the MMTV promoter with a positioned nucleosome (227), and here nucleosome depletion leads to higher accessibility of the promoter for NF-1 binding (228). These findings suggest that the basal repression of the MMTV promoter before hormone induction is due to its organization in chromatin.

Hormone induction was believed to cause a displacement or disruption of the nucleosome over the HRR, manifested by hypersensitivity of this region to DNase I (219, 229), which would facilitate access of NF-1 to its binding site and transcriptional activation (220). Similar nucleosome disruption mechanisms have been described for the glucocorticoid induction of the tyrosine amino transferase gene in rat liver (230), and, in general, the appearance of DNase I hypersensitive sites upon hormone induction is equated with nucleosome displacement. However, in cells carrying a single copy of the MMTV promoter integrated in chromosomal DNA and precisely positioned in a nucleosome, hormone induction does not lead to displacement of this nucleosome, but to a rearrangement which makes only the dyad axis of the nucleosome accessible for double-stranded DNase I cleavage and enables simultaneous binding of receptors, NF-1 and OTF1 (Fig. 3C) (226). As hormone receptors and NF-1 (162), as well as NF-1 and OTF1, compete for binding to naked MMTV DNA (Fig. 3A) (231), our findings suggest that the organization in a nucleosome may be a prerequisite for simultaneous binding of all three factors, and therefore, may contribute to optimal induction (226).

In yeast the MMTV promoter is silent, even in the presence of NF-1, but upon expression of GR and an agonistic ligand, it is activated in an NF-1-dependent fashion (227). Genomic footprinting analysis shows that the promoter is precisely organized in a nucleosome that covers the region between −43 and −195 and that the DNA adopts the same rotational orientation on the surface of this nucleosome as found in animal cells and *in vitro*. After hormone induction there is no indication of displacement or disruption of the nucleosome over the MMTV promoter. In fact, nucleosome depletion leads not only to increased constitutive activity of the MMTV promoter in the absence of receptor and hormone but also to a decreased NF-1-dependent induction in response to hormone (232). These findings support the concept that the nucleosomal organization of the MMTV promoter is involved in both constitutive repression and synergism be-

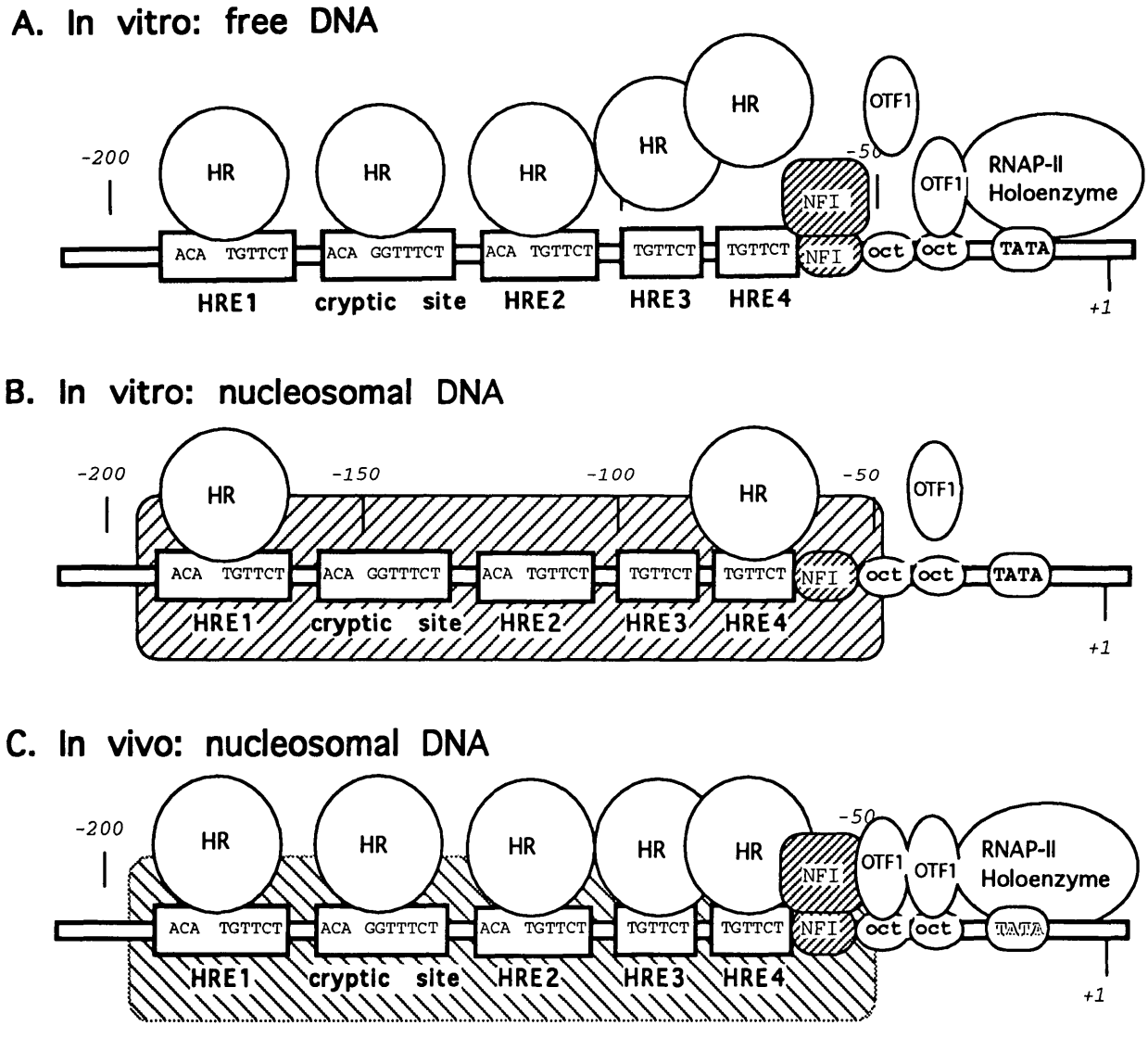

FIG. 3. Occupancy of *cis*-elements on the MMTV promoter *in vitro* and *in vivo*. Schematic representation of the MMTV promoter elements, including HREs, NF-1- binding site, octamer motifs and TATA box. The bound factors are represented as single entities, although in some sites they are known to form homodimers. A. On free MMTV DNA NF-1 competes for binding of hormone receptors (HR) and of OTF1. The cryptic site was described as a region footprinted by the receptors, that when mutated does not exhibit a phenotype in transient transfection (259, 260). B. Binding of factors to the MMTV nucleosome reconstituted *in vitro*. The limits of the nucleosome are indicated by the *elongated rectangle*. Only the accessible HRE1 and 4 are bound, but there is no binding of NF-1 nor of OTF1 to the octamer distal motif (261). C. Binding of factors to the MMTV chromatin in intact cells. All *cis*-elements are occupied simultaneously while the nucleosome remains in place. A possible change in nucleosome structure, documented by appearance of DNASE-I hypersensitivity, is indicated by the different shadowing.

tween receptors and NF-1, which is essential for optimal transcription after hormone induction.

5. Nature of chromatin remodeling. The nature of the hormone-induced conformational rearrangement of chromatin remains obscure, but experiments with inhibitors of histone deacetylases suggest a role for modification of the histone tails. Hyperacetylation of lysine residues in the N-terminal tails of all four core histones is one of the modifications associated with transcriptionally active chromatin (233). It has been found that restriction of GAL4 binding to reconstituted nucleosomes containing GAL4-binding sites can be alleviated by proteolytic digestion of the histone tails, suggesting a general repressive role for these highly charged domains of the core histones (234, 235). Similarly, binding of the transcription factor TFIIIA to a reconstituted nucleosome carrying a 5S RNA gene is enhanced by acetylation of the histone tails (236). In the case of MMTV, complete inhibition of histone deacetylase with 5–10 mM sodium butyrate inhibits hormone induction and nucleosome remodeling (237). However, lower concentrations of butyrate activate hormone-independent transcription from single-copy integrated MMTV reporters (238). A similar response is observed with a more selective inhibitor of histone deacetylase activity, trichostatin A, which acts at nanomolar concentrations (239). Moreover, inducing concentrations of butyrate or trichostatin A generate the same type of DNase I hypersensitivity over the pseudo-dyad axis of the regulatory nucleosome that we observed after hormone induction (232).

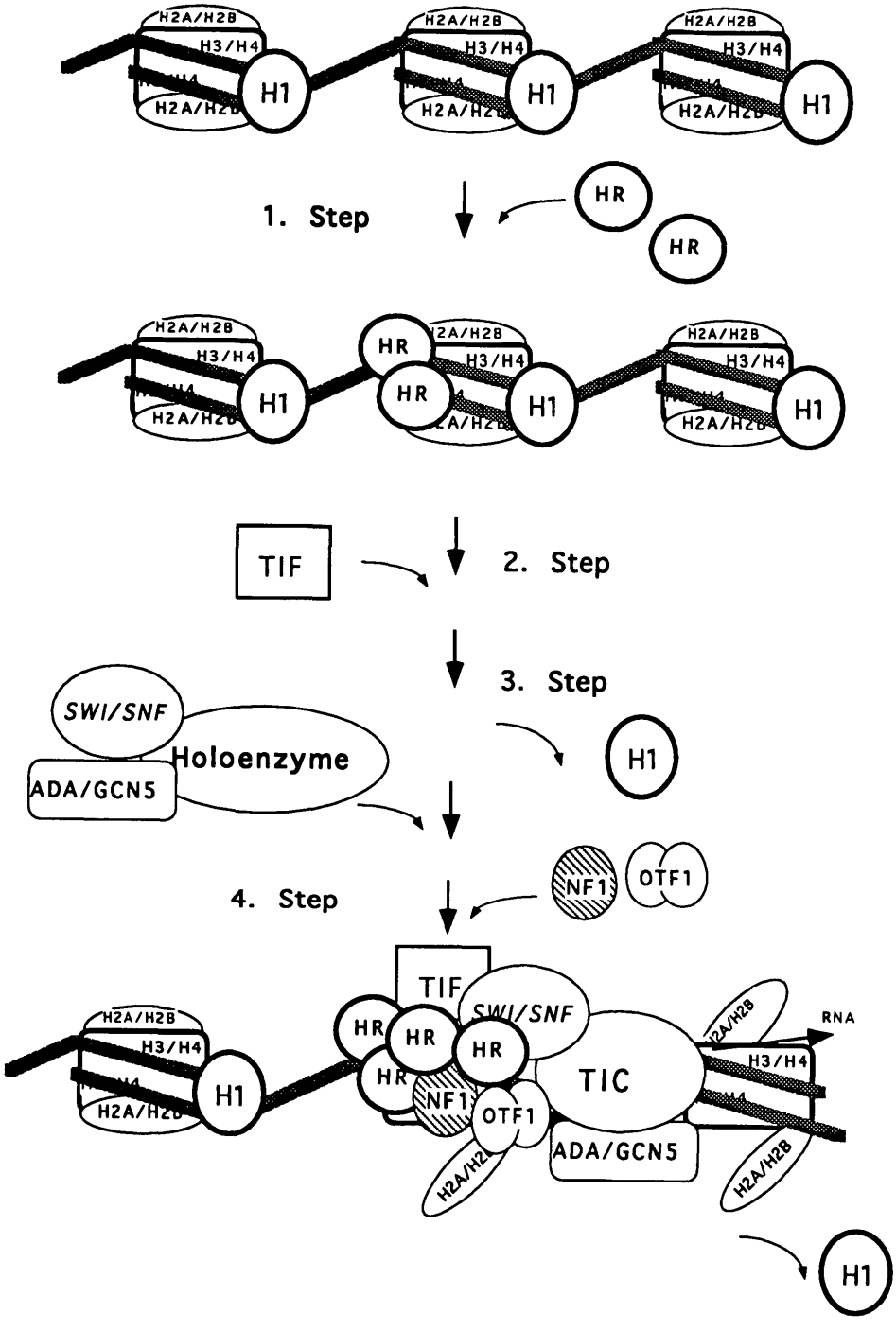

FIG. 4. Multistep chromatin remodelling. A hypothetical model for chromatin remodelling involving several steps. In the first step, hormone receptors (HR) bind to the accessible HREs on the surface of the regulatory nucleosome. In a second step, they recruit, either directly or via transcription intermediary factors (TIF) a holoenzyme complex, or components thereof (for instance SWI/SNF), which participate in remodelling chromatin. Remodelling could involved acetylation of the core histone tails by the ADA/GCN5 complex, removal of histone H1, or/and partial dissociation of a histone H2A/H2B dimer. After these individual remodeling steps, additional receptor molecules would occupy the other HREs and NF-1, as well as OTF1 would enter the promoter. All sequence-specific factors probably cooperate in final recruitment and stabilization of the transcription initiation complex (TIC). Further steps should be considered for efficient promoter release and elongation, which may require participation of still additional factors.

Although we do not know whether hormone treatment alters the acetylation of core histones, these results suggest that a nucleosome remodeling similar to that induced by receptor binding can be generated by changes in the core histone tails.

It is intriguing that the hormone-induced structural alteration in chromatin structure takes place at the nucleosome pseudo-dyad axis, a region shown to be crucial for control of chromatin-mediated gene expression involving the SWI/

SNF complex in yeast (200, 240) (Table 5). Mutations in histones H3 and H4 located near the nucleosome dyad suppress the phenotype of the *swi/snf* mutations (200), suggesting that the architecture of this region of the nucleosome is an important determinant of transcriptional activity. Recently, a soluble complex of all SWI/SNF gene products, which may play an important role in facilitating chromatin transcription, has been identified (241, 242). This complex enhances binding of GAL4 derivatives to nucleosomally organized GAL4-binding sites in an ATP-dependent manner. This effect seems to be accompanied by depletion of histone H2A/histone H2B dimers and is facilitated by nucleoplasmin (243). It is conceivable that hormone-induced chromatin remodeling is accomplished by a receptor-dependent recruitment of the SWI/SNF complex to the MMTV promoter, followed by destabilization of the histone octamer, which would then facilitate access to masked HREs, to the NF-1-binding site, and to the octamer distal motif.

Very recently the SWI/SNF complex has been identified as one of the components of the RNAP-II holoenzyme in yeast (213). Although this finding remains controversial (242), it raises the interesting possibility that some sequence-specific transactivators may recruit the complete machinery required for activated transcription and chromatin remodeling in one single step. It is conceivable that the recruitment mechanism is actually reversed and DNA is recruited to the active sites of transcription. The complex machinery needed for transcription, DNA repair, and RNA splicing could form nuclear compartments stabilized by protein-protein interactions, to which the transactivators would recruit the relevant promoter/enhancer regions on which they act. This would provide a local concentration of the various factors required for the complex chain of reactions involved in gene expression and repair.

However, other mechanisms may also modulate the binding of factors to nucleosomally organized DNA. One attractive possibility is a direct interaction with core histones, similar to that reported between the regulatory proteins SIR3 and SIR4 and the N-terminal regions of histones H3 and H4 in the context of telomere silencing in yeast (244). Recently an interaction between the globular domain of histone H3 and the C-terminal region of CTF1/NF-1 has been detected (245). This kind of interaction could offer a mechanistic explanation for the positive participation of the nucleosome in mediating synergism between the hormone receptors and NF-1, if the remodeling of the nucleosome induced by the ligand-activated receptor led to the exposure of the NF-1-interacting surface of histone H3. However, this model seems unlikely as the NF-1 variant used for most of our studies lacks the region of the transactivation domain responsible for interaction with histone H3.

Recently, a new complex involved in remodeling of chromatin on the heat shock genes has been identified and characterized in *Drosophila* extracts (246). Although this complex, called NURF (Nucleosome Remodelling Factor), is different from the SWI/SNF complex, it contains a subunit, ISWI, which is a member of the SWI2/SNF2 class of ATPase (247). Curiously, the ATPase activity of NURF is stimulated by nucleosomes rather than naked DNA, which stimulates the ATPase activity of the SWI/SNF complex. Additional ATP-dependent remodeling activities have been described in *Drosophila* embryo extracts (217, 248), but their characterization is not yet completed.

In an electron microscopic study, transcription of reconstituted nucleosomal templates by T7 RNAP in a defined *in vitro* system disrupts nucleosomes in the transcribed region, while nucleosomes are preserved upstream of the initiation site and in front of the polymerase (249). In contrast, transcription of the vitellogenin B1 gene by RNAP-II or T7 polymerase in liver nuclear extracts preserves nucleosome structure. The responsible nuclear activity (called TCSA-1, transcription-dependent chromatin stabilizing activity I) is also present in HeLa cells, is heat labile, and cannot be replaced by nucleoplasmin or other known factors (249). The emerging picture is that the cell devotes a considerable fraction of its genetic information to code for various chromatin remodeling complexes and that much more will be uncovered in this field during the next years.

A new class of histone H2A mutants causes defects in regulated gene expression but does not affect all SWI/SNF-dependent genes (250). Suppressors of SWI/SNF, such as Spt6, do not suppress these H2A mutations, some of which are semidominant and located close to the N- and C-terminal regions. In mutant strains, the SUC2 promoter is in an active chromatin conformation, as if the mutated histones are suitable for nucleosome assembly but not for transcriptional activation. These results suggest the existence of a second step in chromatin remodeling distal to the action of the SWI/SNF complex (250). An interesting candidate component of this activity is Hpr1, a gene that, when mutated, generates a hyperrecombination phenotype (251). Most of Hpr1 protein is in a large complex distinct from the SWI/SNF. It may act by altering chromatin structure since simultaneous increase in gene dosage of all four core histones suppresses the phenotype of *hpr1* mutants (252). In addition, *hpr1* mutants are defective in induced gene expression, and mutations in the SRB2 gene suppress the *hpr1* phenotype (253).

As the linker histones are important for condensation of the 10-nm chromatin fiber (254), they could also influence the accessibility of DNA sequences in chromatin. Moreover, important *cis*-elements of the MMTV promoter are located at the edge of the positioned nucleosome or in the linker DNA, which are regions contacted by histone H1 (255). It has been stated that upon hormone induction there is a depletion of histone H1 from the MMTV promoter, and this could influence the accessibility of promoter sequences (256), possibly through their effect on nucleosome mobility (257). Although this remains an attractive possibility, it cannot be an essential prerequisite for hormonal induction of MMTV transcription, since the MMTV promoter is perfectly regulated in yeast, which lacks linker histones (227). It is possible that in metazoan cells linker histones potentiate the induction process by generating a stronger repression in the absence of transactivators. The emerging picture is still poorly defined, but it is clear that chromatin remodeling is an essential step in gene regulation that involves a large number of activities and multiple protein-protein interactions, which we are only beginning to unravel. A completely hypothetical model of how

these various processes could take place on the MMTV promoter is proposed in Fig. 4.

V. Conclusions

During the past few years we have made considerable progress in defining the components of the transcription initiation complex and the mechanism involved in promoter clearance. We now know that coordinate interaction of a large number of GTFs is required for the correct initiation of transcription, and that regulated phosphorylation steps are involved in controlling the transition of the initiation complex into an elongation complex. However, we still do not know to what extent the transcription initiation complex is assembled in a sequential and stepwise fashion on the promoter, and to what extent a preformed holoenzyme is recruited by sequence-specific transactivators. In this case, however, the complex will have to assemble somewhere else, or be the product of a previous sequential assembly, which can detach from the template as a unit. Moreover, the exact composition of the holoenzyme complex remains controversial, and it is not unlikely that many different complexes with a common core but different additional components exist within the cell nucleus.

The interaction of steroid hormone receptors with the GTFs and TAF_{II}s can take place directly, although these contacts are not very efficient. A large and still growing family of intermediary factors has been discovered in the last 2 yr, some of which seem to mediate the interaction of the receptors with GTFs, while others may participate in the receptor-mediated chromatin remodeling.

The role played by chromatin remodeling in the regulated assembly of the transcription initiation complex and in promoter clearance is revealing completely new biochemical activities of unpredicted complexity. Certainly the field of energy-dependent chromatin remodeling and its relationship to gene expression will occupy a central role in the field of transcriptional regulation for the years to come and will profit from the combination of yeast genetics and biochemical studies with reconstituted chromatin.

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