Common themes in the function of transcription and splicing enhancers

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Regulation of both transcription and RNA splicing requires enhancer elements, that is, *cis*-acting DNA or RNA sequences that promote the activities of linked promoters or splice sites, respectively. Both types of enhancer associate with regulatory proteins to form multicomponent enhancer complexes that recruit the necessary enzymatic machinery to promoter or splice site recognition sequences. This recruitment occurs as a result of direct interactions between regulatory proteins in the enhancer complexes and components of the basic enzymatic machineries. Recent advances suggest that the high degree of regulatory specificity observed for both transcription and splicing is due, in large part, to the multicomponent nature of enhancer complexes and to their cooperative assembly.

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Abbreviations

BPS	branchpoint sequence
dsx	*doublesex*
GTF	general transcription factor
hnRNP	heterogeneous ribonucleoprotein
Inr	initiator element
$(Py)_n$	pyrimidine tract
polII	RNA polymerase II
snRNP	small nuclear ribonucleoprotein particle
SR	serine–arginine-rich
TBP	TATA-binding protein
TFII	transcription factor II
Tra	transformer
U2AF	U2 snRNP auxiliary factor

Introduction

The mechanisms for regulating transcription and splicing require special DNA and RNA elements, respectively, which are termed enhancers. Transcription enhancers are recognized by specific activator proteins, whereas most splicing enhancers are bound by members of a family of serine–arginine-rich (SR) proteins. Both transcription and splicing activator proteins have in common a modular organization, with separable nucleic acid binding domains and regions required for specific protein–protein interactions.

Although the detailed mechanisms of enhancer function are not understood for either transcription or splicing, some common features of the two processes have been identified. In both cases, cooperative binding of regulatory proteins to enhancers leads to the formation of highly stable complexes that recruit the basic components of the transcription or splicing apparatus to nearby recognition elements. Here, we review recent advances that have revealed similarities in the regulation of transcription and splicing, including the multicomponent nature of enhancer complexes, the modular organization of enhancer-binding proteins, the enhancer-dependent recruitment of general transcription or splicing factors and the roles of cooperativity and synergy. We begin by introducing these phenomena in the context of transcription enhancers, and then discuss similarities and differences in the regulation of transcription and splicing.

Transcription enhancers

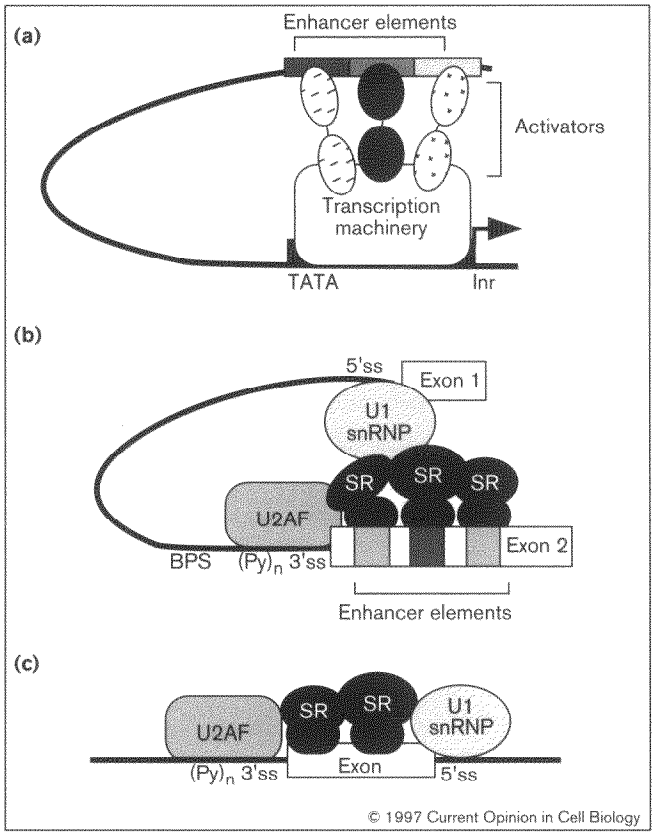
The RNA polymerase II holoenzyme

The basal promoter, consisting of a TATA box and/or an initiator element (Inr) (Fig. 1a), is sufficient for low levels of accurately initiated transcription *in vitro* and when introduced into cells by transfection. The basal promoter is recognized by RNA polymerase II (polII), several general transcription factors (GTFs), including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, and other components of the 'transcription machinery' [1]. Although these proteins assemble on the promoter in a stepwise fashion *in vitro*, high molecular weight complexes containing polII, several GTFs and additional transcription components can be isolated from both yeast [2,3] and mammalian cells [1,4••,5]. These latter complexes, referred to as the 'holoenzyme', may be recruited to the promoter in a single step by linked enhancer elements [3].

The architecture of transcription enhancers

Transcription enhancers can function at considerable distances from the promoter, and are active upstream of, within, or downstream of the gene, regardless of orientation with respect to the promoter [6]. Moreover, transcription enhancers vary in complexity, from simple elements containing one or more binding sites for a single type of activator protein, to enhancers containing binding sites for several different activators [7,8•]. Activator proteins contain at least two functional modules, that is, a separable DNA-binding domain and an activation domain, the latter of which contacts proteins in the transcriptional machinery. Some enhancers also bind proteins that lack an activation domain but function to induce DNA bending and promote protein–protein interactions within the enhancer complex [9•,10•,11]. Genes containing these enhancers are transcribed at high levels only when the appropriate set of activator proteins is present and a specific higher-order structure is assembled [9•].

Figure 1



Recruitment model for transcription **(a)** and splicing **(b)** enhancers. (a) Transcription of eukaryotic genes is initiated at promoter elements consisting of an AT-rich region called the TATA box, located 30 base pairs upstream of the start site, and/or an Inr that directly overlaps the start site. The promoter functions as a binding site for the transcription machinery. Transcriptional activator proteins bind to the enhancer and recruit the transcription machinery to the promoter through protein–protein interactions. The thick black line in (a) represents double-stranded DNA. The arrow represents transcription initiation. (b) Components of the splicing machinery (U2AF and U1snRNP) recognize splice-site signals, that is, a consensus sequence at the 5′ splice site (5′ss; nucleotide sequence YRG/GURRGU), the branchpoint sequence (BPS; sequence YNRAY), a pyrimidine-rich tract $(Py)_n$ and the 3′ intron/exon junction (YAG/N) at the 3′ splice site (3′ss), and exonic enhancer sequences (N=A, C, G, U; Y=C, U; R=A, G). The splicing machinery is assembled on the intron (thick black line) in a stepwise fashion, and during initial spliceosomal complex (E complex) formation U1snRNP and U2AF bind to the 5′ and 3′ splice sites, respectively. SR proteins bind to specific RNA sequences (enhancer elements) in the splicing enhancer and recruit U2AF and U1snRNP. **(c)** SR proteins can also mediate interactions between U1snRNP and U2AF that are bound to either side of an exon, thus facilitating recognition of a weak 3′ splice site. Black lines represent intronic RNA. Transcriptional activators (a) and SR proteins (b) have a modular organization, with DNA- or RNA-binding domains and regions required for specific protein–protein interactions.

The recruitment model of enhancer function

A well documented function of enhancer complexes is to recruit the transcription machinery to linked promoters through direct protein–protein interactions between the activators and one or more components of the transcriptional machinery (Fig. 1a) [12••]. Interactions between the transcription machinery and activators are thought to stabilize the binding of GTFs to the basal promoter. Thus, enhancers increase the formation of productive pre-initiation complexes [12••,13•]. This mechanism may involve recruitment of the holoenzyme [14••], or, alternatively, the recruitment and assembly of individual GTFs in a stepwise fashion [1]. Components of the transcription machinery that interact with activators are known as activator targets. Many such targets have been identified, and evidence that these interactions are required for transcriptional activation is discussed in detail elsewhere [12••,15].

In the recruitment model, activators function by binding to the enhancer and directing the transcription machinery to the basal promoter. Thus, activators do not induce conformational changes in the transcription machinery, nor do they promote any enzymatic activity. Evidence for this model has recently been strengthened by the observation that a single noncovalent interaction between a protein bound to the enhancer and a holoenzyme component can suffice for enhancer function [14••]. However, in the context of natural promoters, high levels of transcriptional activity appear to require multiple weak interactions between activators bound to the enhancer and components of the transcription machinery [7].

Additional evidence for recruitment is provided by the analysis of recombinant proteins consisting of a heterologous DNA-binding domain fused either to a GTF such as the TATA-binding protein (TBP) [16••] or to a holoenzyme component [14••,17]. In both cases, the binding of the fusion protein to a site upstream from a promoter results in high levels of transcription activation. A final argument for recruitment is provided by the observation that the strength of individual activator–target interactions is directly proportional to the level of transcription enhancement [18•]. Taken together, these observations strongly argue that a primary function of the enhancer is to recruit the transcription machinery to the promoter. However, it is important to note that these arguments are based primarily on the analysis of simple promoters and artificial activators. Thus, the possibility that postrecruitment interactions between activators and the transcription machinery occur in a natural enhancer–promoter context cannot be excluded. For instance, certain activators have been shown to increase the processivity of a recruited polII [19,20•].

Enhancers counteract chromatin repression

A number of enhancer-dependent mechanisms have been described for overcoming gene repression by chromatin, which renders promoter sequences less accessible to the transcription machinery [21]. For example, transcriptional activators can recruit histone acetylase activity to promoters [22,23••]. This activity neutralizes the positive charge

on histones, thus weakening histone–DNA interactions. Activator proteins can also recruit chromatin-remodeling complexes such as Swi/Snf [24] or NURF (nucleosome remodeling factor) [25] to the gene. Interestingly, the Swi/Snf complex may be recruited to the promoter by virtue of its association with the holoenzyme complex [26•]. Although the Swi/Snf complex is required for maximal activity from certain promoters, in at least some cases recruitment of a holoenzyme lacking a functional Swi/Snf complex appears to be sufficient for chromatin remodeling [27]. Thus, the requirement for specialized chromatin-remodeling activities may depend on the strength of the promoter.

Transcriptional synergy

Studies of simple enhancer elements containing binding sites for identical or different transcription factors revealed that an increase in the number of transcription factor binding sites results in a greater than additive (i.e. synergistic) increase in transcription [13•,28,29,30•,31•,32–35]. As illustrated in Figure 2a, some synergistic effects have been correlated with the cooperative binding of activators to multiple sites [32,33]. Transcriptional synergy can also be observed *in vitro* under conditions in which the binding sites are fully occupied by the activator [28,29,30•,31•,34,35]. Under these saturating conditions, if only one activator interacts with the transcription machinery at a time, the multimerization of enhancer elements would result in an additive increase in activation, proportional to the number and strength of the enhancer-bound activators present (Fig. 2b). The synergistic activation by enhancers therefore suggests that activation is stimulated by multiple, simultaneous interactions between the bound activators and distinct components of the transcription machinery (Fig. 2c) [12••,28,29,30•,31•,34,35]. Furthermore, in some cases synergy may arise from the enhancement of two or more inefficient steps following recruitment of the transcription machinery [19,20•,36]. We should note, however, that the detection of synergy can reach a limit if the presence of a single, strong activator is sufficient to trigger maximal stimulation. Thus, if the protein–protein and protein–DNA interactions of an activator are sufficiently strong no cooperativity is observed [32,34].

Cooperativity fulfills an important biological requirement for transcriptional regulation. On the one hand, it allows for highly specific gene activation via a combinatorial mechanism, as, in a typically complex enhancer containing multiple binding sites, maximal activity is achieved only when all of these sites are filled [7,8•,10•]. On the other hand, as the activation of a particular gene is highly sensitive to the presence of all necessary factors, small changes in the concentration of a single limiting factor can lead to a highly sensitive on/off switch [8•].

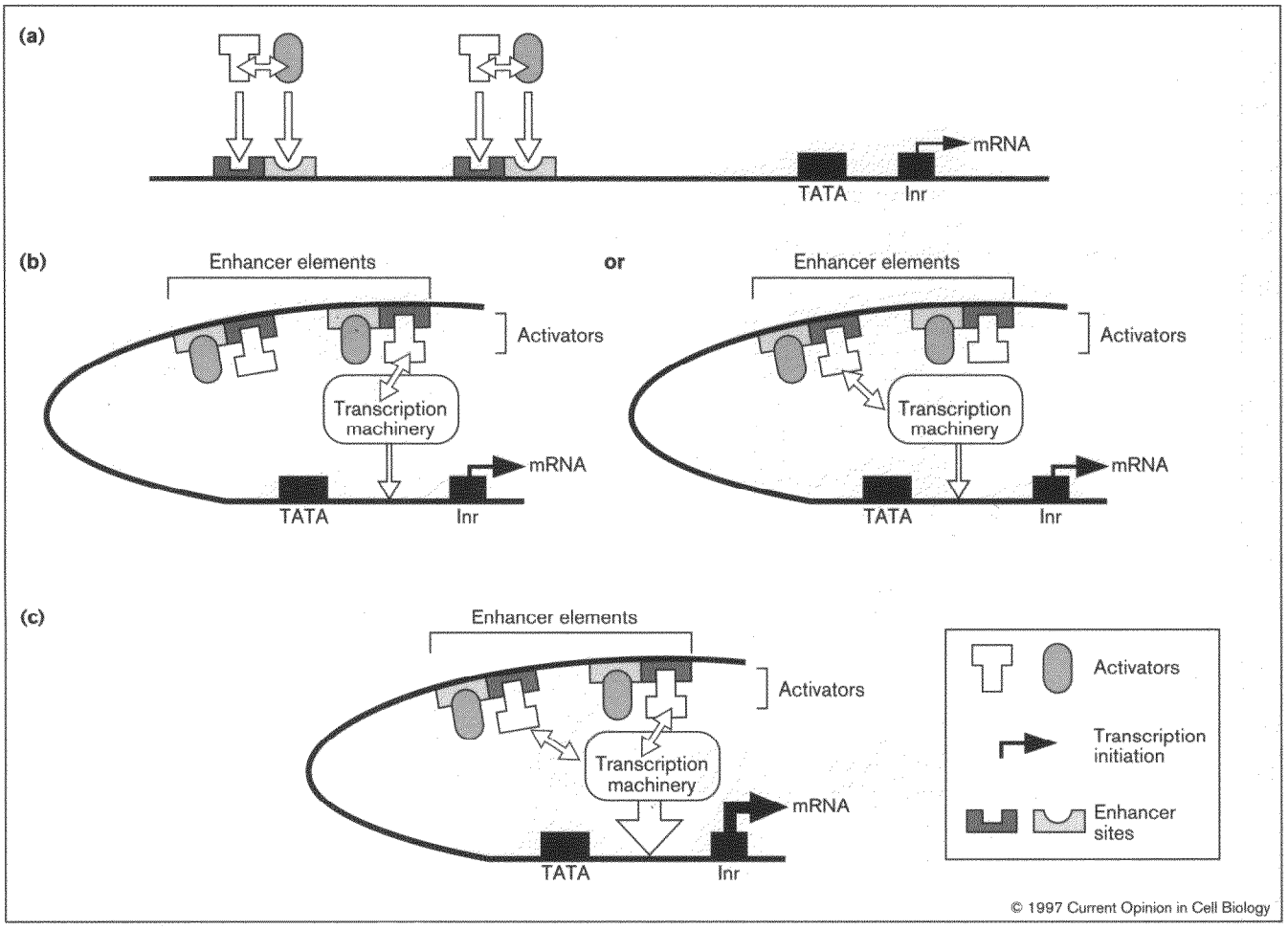
Splicing enhancers

Although the enzymatic machineries required for transcription and splicing are unrelated, the mechanisms by which they are directed to specific sites of action may be similar. The precise removal of introns from eukaryotic pre-messenger RNAs (pre-mRNAs) requires the recognition of splice junctions and the stepwise assembly of high molecular weight spliceosomal complexes consisting of over 50 different proteins and five small nuclear RNAs (snRNAs) [37,38]. In higher eukaryotes, the RNA elements required for splice-site recognition include the sequences at the 5′ and 3′ splice sites in addition to sequences at the lariat branchpoint sequence (BPS) and the pyrimidine tract ($[Py]_n$) (Fig. 1b). Early attempts to identify sequences required for accurate splice-site selection also revealed an important role for exon sequences [39,40•]. Subsequently, specific exon sequences capable of strongly stimulating weak splice sites in adjacent introns were discovered and designated splicing enhancers, because of their ability to act on heterologous splice sites [41,42]. Most splicing enhancers are located within 100 nucleotides of the regulated splice site, although certain enhancers have been shown to function at distances of up to at least 500 nucleotides [43]. The majority of splicing enhancers are located downstream from, and regulate, weak 3′ splice sites; however, some have been observed within introns, and others are thought to influence the strength of the BPS or the 5′ splice site [42,43].

Specific members of the SR protein family of splicing factors bind to splicing enhancers and are required for their activities [40•,43–45]. Like transcriptional activator proteins, SR proteins are modular, containing one or more RNA-binding domains and an SR domain [44,45] which is required for protein–protein interactions [46,47]. In at least one case, a fusion protein, in which an SR domain was fused to a heterologous RNA-binding domain, was capable of promoting splicing, thus suggesting that these two domains are functionally separable [48]. A number of other proteins also contain SR domains, including spliceosomal components involved in initial splice-site recognition (namely U1snRNP [U1 small nuclear ribonucleoprotein particle] and U2AF [U2 snRNP auxiliary factor]) [40•,44,45]. SR proteins interact with one another, with U1snRNP and with U2AF through their SR domains [46,47]. Evidence that these interactions are required for splicing enhancer function is provided by the observation that the binding of SR proteins to an enhancer strongly promotes the binding of U2AF to an upstream weak 3′ splice site [49•]. Similarly, enhancer-bound SR proteins may activate weak 5′ splice sites through recruitment of U1snRNP [47,50].

Thus, as for transcription enhancers, the function of splicing enhancers is to stabilize the interactions between

Figure 2



Models for transcriptional synergy. Thick black lines represent DNA. Elements of the promoter (the TATA box and the Inr) are shown. Synergy can occur at the level of activator binding to the enhancer's binding sites **(a)** and/or at the level of communication between enhancer-bound activators and the transcription machinery **(c)**. (a) Cooperative binding of activators to the enhancer. The protein–protein interactions between two nonidentical (as shown here) or identical activators increases their affinities for the enhancer. Thus, the sum of protein–DNA and protein–protein interactions stabilizes the enhancer–activator complex. **(b,c)** Multiple identical or nonidentical enhancer complexes interact with the transcription machinery. (b) If only one target within the transcription machinery is available for the activator complex at a time, multiple enhancer sites have only an additive effect on transcriptional activation. (c) In contrast, if both enhancer complexes participate in transcriptional activation either by simultaneously interacting with two targets or by increasing the rate of two or more slow steps in the process of transcriptional initiation and/or elongation, transcriptional synergy is observed. Of the open arrows, double-headed ones represent interactions, and single-headed ones represent binding. Larger arrows indicate larger effects than do smaller arrows.

splicing components and splice site recognition signals (Fig. 1b). This principle is dramatically illustrated by the observation that 5′ and 3′ splice sites located on separate RNA molecules can be spliced in *trans* if a splicing enhancer is located downstream from the 3′ splice site [51•,52•]. Furthermore, this enhancement of *trans*-splicing is dependent on the presence of SR proteins [52•]. Thus, the complex formed between the enhancer-bound SR proteins and 3′ splice site components is sufficiently stable to allow a bimolecular reaction between 5′ and 3′ splice sites.

In another analogy to transcription enhancers, splicing enhancers can range in complexity from a simple site to an array of identical or nonidentical binding sites [41–43,53]. In fact, functional enhancer elements can be selected at a high frequency from random RNA sequences ([54]; T Schaal, T Maniatis, unpublished data), and these sequences are capable of binding specific SR proteins ([55•]; T Schaal, T Maniatis, unpublished data) and promoting splice-site utilization ([54,55•]; T Schaal, T Maniatis, unpublished data). Furthermore, as observed for transcription, the complexity of a splicing enhancer is roughly proportional to the strength of its activity, and thus to the distance from the intron (or the promoter in transcription) at which it can function [53,56,57]. In both cases, this relationship between complexity and strength is probably due to a synergistic increase in the level of activation, either derived from cooperative binding of activators to the enhancer or from multiple interactions

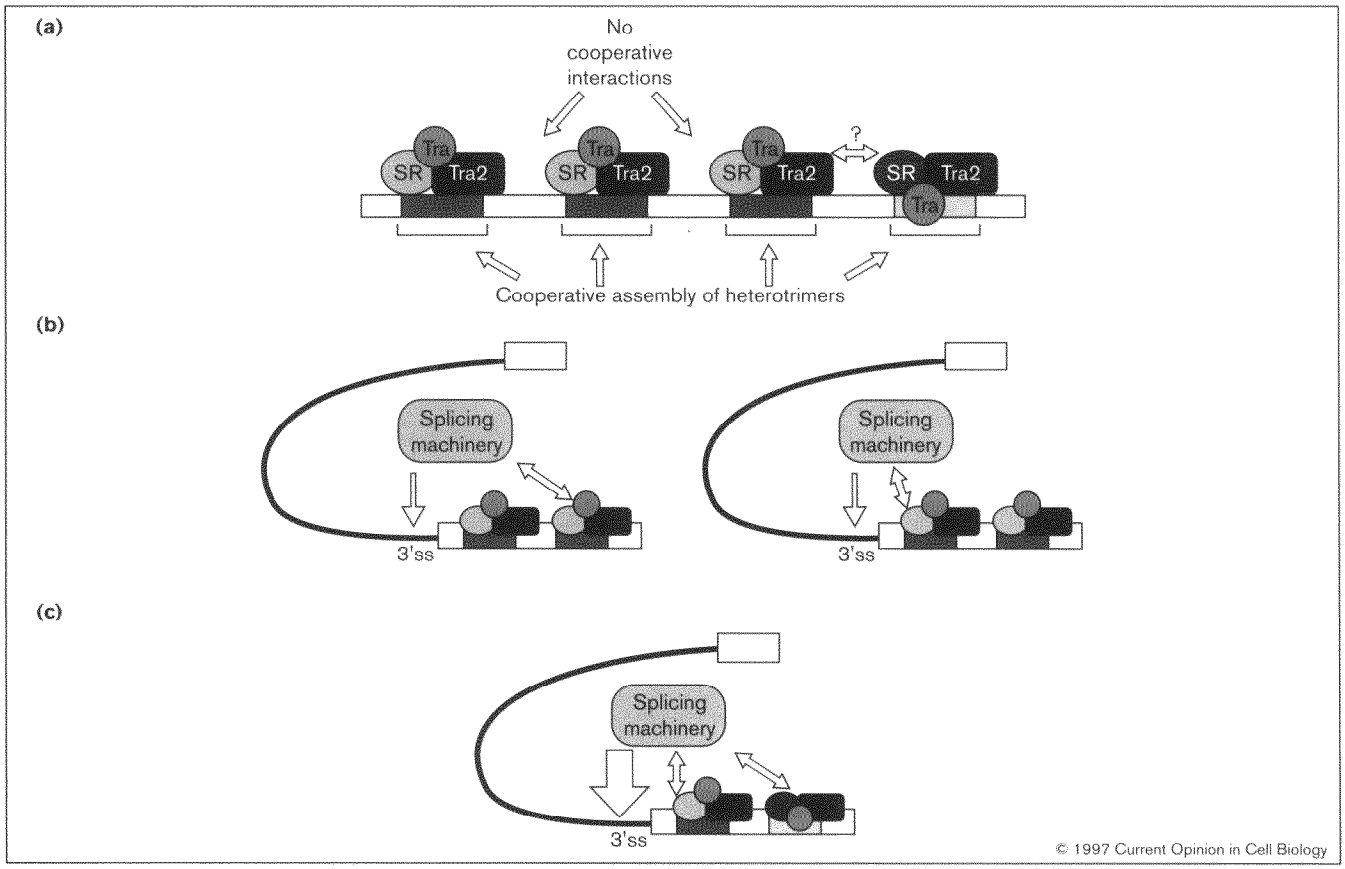
between the enhancer complex and the basic splicing or transcription machinery.

Splicing synergy

As observed for the assembly of a number of transcriptional activator complexes, the binding of SR proteins to at least some splicing enhancer elements is highly cooperative (Fig. 3a) [58••]. The best characterized example of this is seen in the enhancer complex that activates the weak female-specific 3′ splice site in *Drosophila doublesex* (*dsx*) pre-mRNA. The *dsx* splicing enhancer contains multiple binding sites located approximately 300 nucleotides downstream of the regulated 3′ splice site, and requires SR proteins and the splicing regulators Tra (transformer) and Tra2 for activity [59–62]. Each binding site associates with a complex of the three proteins, that is, Tra, Tra2, and a specific SR protein. Specific interaction of each of these proteins with the RNA sequence is highly dependent on the presence of the other proteins (Fig. 3a) [58••]. Although the *dsx* enhancer provides the best characterized example of such cooperativity, initial studies with other enhancers have suggested the existence of similar mechanisms [63•,64].

The cooperative binding of SR proteins provides a basis for a combinatorial mechanism for specific RNA sequence recognition. Individual SR proteins display only weak specificity for their binding sites [44,45,53]. However, cooperative binding of homo- or heterodimeric SR proteins could lead to the specific recognition of a wide range of different RNA sequences. Cooperativity between SR proteins, U2AF, and U1snRNP is also likely to play a significant role in the selection of splice sites in relatively short exons [40•,65•]. A number of studies have shown that

Figure 3



Models for synergy of splicing enhancers. Thick black lines represent intronic RNA. **(a)** The formation of heterotrimeric complexes on the two distinct RNA sequence elements (represented by differently shaded gray rectangles) of the *dsx* splicing enhancer is dependent on cooperative protein–protein and protein–RNA interactions. In contrast, no cooperativity is observed between the identical complexes which bind to the repeat sequences (dark gray shaded rectangles). It is not yet known whether cooperativity exists between the complexes that bind to the repeats and the complexes that bind to the purine-rich element (PRE; light gray shaded rectangles). **(b)** Multiple repeat-specific complexes function additively. This suggests that the target in the splicing machinery can interact with only one of these complexes at a time. 3′ss, 3′ splice site. **(c)** The synergistic activity seen when the PRE is present together with the repeats may indicate that the complexes bound to these distinct sequences can simultaneously recruit the splicing machinery. Alternatively, this synergistic behavior may result from cooperative binding as described in (a). Larger arrows represent greater splicing activity than do smaller arrows.

a downstream 5′ splice site can enhance the recognition of a weak upstream 3′ splice site [40•,51•,65•], and it is likely that this enhancement is dependent on the presence of SR proteins [40•]. Although SR proteins in isolation may associate only weakly with exonic sequences, in the presence of a 5′ splice site and U1snRNP the SR proteins may interact with both U1snRNP and U2AF, thus forming a 'bridge' between the 5′ and 3′ splice sites (Fig. 1c) [40•,65•].

By analogy to the approach taken to elucidate whether synergistic interactions exist between activators and the transcription machinery, splicing enhancers were tested at saturated protein concentrations. In the case of *dsx*, the presence of multiple sites (referred to as the repeat elements) that bind identical protein complexes results in an additive, rather than a synergistic, increase in the level of splicing (KJ Hertel, T Maniatis, unpublished data). Thus, in contrast to transcription where synergy is observed for both identical and nonidentical activators, the additivity observed for multiple copies of the *dsx* repeat element suggests either that only one enhancer complex at a time interacts with a single spliceosomal target (Fig. 3b) or that a single interaction between the enhancer complex and the 3′ splice site is sufficient for maximal levels of splicing.

Despite the observation of additivity with identical enhancer elements, it is possible that multiple non-identical enhancers might have a synergistic effect on spliceosomal recruitment if their targets are mutually exclusive (Fig. 3c). For example, the synergy observed in the *dsx* enhancer in the combined activity of the two types of enhancer element (the repeat elements and a purine-rich element) [53] might constitute such a case. However, this observation was made only at very low concentrations of Tra and Tra2. Therefore, this synergy may result either from cooperative binding to the enhancer (Fig. 3a), from cooperative recruitment of the spliceosomal machinery (Fig. 3c), or from both processes.

A final consideration is that splice site recognition sequences may be blocked by a family of RNA-binding proteins known as hnRNPs (heterogeneous ribonucleoproteins). These proteins associate to form hnRNP particles that are arranged along RNA like beads on a string [66,67], in a manner that is similar to the packaging of DNA by nucleosomes. When pre-mRNAs are added to an *in vitro* splicing reaction, hnRNPs rapidly associate to form a nonfunctional H complex which may subsequently be chased into the earliest prespliceosomal E complex [38]. Thus, one function of splicing enhancers and SR proteins may be to remove hnRNPs from critical splice site recognition sequences [38,68]. Further studies are required to confirm whether the hnRNP complex might be the target of enhancer-directed regulation.

Conclusions

In summary, transcription and pre-mRNA splicing are distinct processes, but they are regulated by similar mechanisms. In both cases, highly specific enhancer complexes, assembled through cooperative weak interactions, are responsible for the efficient recruitment of the transcription or splicing machinery. Thus, it appears that synergy, however accomplished, is a general mechanism for achieving the sensitive regulation required for many biological processes.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Orphanides G, Lagrange T, Reinberg D: **The general transcription factors of RNA polymerase II.** *Genes Dev* 1996, **10**:2657–2683.

2. Koleske AJ, Young RA: **An RNA polymerase II holoenzyme responsive to activators.** *Nature* 1994, **368**:466–469.

3. Koleske AJ, Young RA: **The RNA polymerase II holoenzyme and its implications for gene regulation.** *Trends Biochem* 1995, **20**:113–114.

4. •• Chao DM, Gadbois EL, Murray PJ, Anderson SF, Sonu MS, Parvin JD, Young RA: **A mammalian SRB protein associated with an RNA polymerase II holoenzyme.** *Nature* 1996, **380**:82–85.

SRB proteins (suppressors of RNA polymerase B) are essential components of the yeast holoenzyme, originally identified by genetic suppression of polII truncation mutations. This paper shows that a mammalian polII holoenzyme containing polII, TFIIE and TFIIH can be isolated using antibodies against SRB7, a human homolog of yeast SRB proteins. Thus, both the yeast and mammalian polII holoenzymes can be identified, and both contain SRB proteins.

5. Maldonado E, Shiekhattar R, Sheldon M, Cho H, Drapkin R, Rickert P, Lees E, Anderson CW, Linn S, Reinberg D: **A human RNA polymerase II complex associated with SRB and DNA-repair proteins.** *Nature* 1996, **381**:86–89.

6. Maniatis T, Goodbourn S, Fischer JA: **Regulation of inducible and tissue-specific gene expression.** *Science* 1987, **236**:1237–1245.

7. Tjian R, Maniatis T: **Transcriptional activation: a complex puzzle with few easy pieces.** *Cell* 1994, **77**:5–8.

8. • Hill CS, Treisman R: **Transcriptional regulation by extracellular signals: mechanisms and specificity.** *Cell* 1995, **80**:199–211.

A comprehensive review describing how combinatorial interactions allow for the transcriptional activation of specific genes in response to various external stimuli.

9. • Grosschedl R: **Higher-order nucleoprotein complexes in transcription: analogies with site-specific recombination.** *Curr Opin Cell Biol* 1995, **7**:362–370.

An excellent minireview describing the role of architectural proteins and DNA bending in the function of transcriptional enhancers.

10. • Thanos D, Maniatis T: **Virus induction of human IFN-β gene expression requires the assembly of an enhanceosome.** *Cell* 1995, **83**:1091–1100.

The authors of this paper demonstrate a role of the architectural protein HMG I(Y) in enhancer function, and show that cooperative binding of

transcription activator proteins *in vitro* and transcriptional synergy *in vivo* require the precise positioning of transcription factor binding sites on the face of the DNA helix. Thus, at least some enhancers may form higher-order multiprotein complexes termed enhanceosomes.

11. Falvo JV, Thanos D, Maniatis T: **Reversal of intrinsic DNA bends in the IFN-β gene enhancer by transcription factors and the architectural protein HMG I(Y).** *Cell* 1995, **83**:1101–1111.

12. •• Ptashne M, Gann A: **Transcriptional activation by recruitment in bacteria and yeast.** *Nature* 1997, **386**:569–577.
A comprehensive and critical review summarizing recent progress in understanding the mechanisms of transcriptional activation.

13. • Pugh BF: **Mechanisms of transcription complex assembly.** *Curr Opin Cell Biol* 1996, **8**:303–311.
This review discusses the mechanisms involved in gene activation, including enhancer accessibility, recruitment of TFIID and polII, and transcriptional synergy. These processes are discussed in the context of the stepwise or holoenzyme recruitment models.

14. •• Barberis A, Pearlberg J, Simkovich N, Farrell S, Reinagel P, Bamdad C, Sigal G, Ptashne M: **Contact with a component of the polymerase II holoenzyme suffices for gene activation.** *Cell* 1995, **81**:359–368.
The Gal11 protein is a component of the yeast holoenzyme. This paper shows that a fortuitous interaction between a mutant of the Gal11 protein and a fragment of the transcriptional activator protein Gal4 suffices for transcriptional activation. Thus, a single contact between a DNA-bound protein and the holoenzyme is sufficient for transcriptional activation, supporting the view that the primary function of activators is to recruit the holoenzyme to the promoter.

15. Ranish JA, Hahn S: **Transcription: basal factors and activations.** *Curr Opin Genet Dev* 1996, **6**:151–158.

16. •• Chatterjee S, Struhl K: **Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain.** *Nature* 1995, **374**:820–822.
This paper shows that the requirement for an activation domain can be bypassed by covalently joining a DNA-binding domain and a component of the transcription machinery (i.e. TBP). In addition, the results show that recruitment of TBP to the promoter can be a rate-limiting step for transcription *in vivo*.

17. Farrell S, Simkovich N, Wu Y, Barberis A, Ptashne M: **Gene activation by recruitment of the RNA polymerase II holoenzyme.** *Genes Dev* 1996, **10**:2359–2367.

18. • Wu Y, Reece RJ, Ptashne M: **Quantitation of putative activator target affinities predicts transcriptional activating potentials.** *EMBO J* 1996, **15**:3951–3963.
Deletion and point mutation variants of a yeast transcriptional activation domain are tested for their affinities to TBP and TFIIB and for their transcriptional activities *in vivo*. A remarkable correlation between affinity and activity was observed, providing strong evidence that TBP and TFIIB are activator targets.

19. Bentley DL: **Regulation of transcriptional elongation by RNA polymerase II.** *Curr Opin Genet Dev* 1995, **5**:210–216.

20. • Blau J, Xiao H, McCracken S, O'Hare P, Greenblatt J, Bentley D: **Three functional classes of transcriptional activation domains.** *Mol Cell Biol* 1996, **16**:2044–2055.
This paper shows that distinct activator domains can function at different stages of transcription *in vivo*, some at the level of initiation, others at the level of elongation. Thus, transcriptional synergy could result, at least in part, by stimulating different steps of transcription.

21. Kingston RE, Bunker CA, Imbalzano AN: **Repression and activation by multiprotein complexes that alter chromatin structure.** *Genes Dev* 1996, **10**:905–920.

22. Brownell JE, Allis CD: **Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation.** *Curr Opin Genet Dev* 1996, **6**:176–184.

23. •• Bannister AJ, Kouzarides T: **The CBP co-activator is a histone acetyl-transferase.** *Nature* 1996, **384**:641–643.
CBP (CREB-binding protein) is a protein that was originally found to specifically associate with activated CREB (cAMP response element binding protein) proteins, but which was subsequently found to associate with, and stimulate the activities of, several transcriptional activator proteins. CBP was also associated with a histone acetylase, P/CAF (p300/CBP-associated protein). The authors of this paper show that CBP has intrinsic histone acetylase activity. Thus, one function of activator proteins is to recruit histone acetylases to the promoter, resulting in a weakening of histone–DNA interactions.

24. Cote J, Quinn J, Workman JL, Peterson CL: **Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex.** *Science* 1994, **265**:53–60.

25. Tsukiyama T, Wu C: **Purification and properties of an ATP-dependent nucleosome remodeling factor.** *Cell* 1995, **83**:1011–1020.

26. • Wilson CJ, Chao DM, Imbalzano AN, Schnitzler GR, Kingston RE, Young RA: **RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling.** *Cell* 1996, **84**:235–244.
The authors of this paper show that components of the yeast Swi/Snf chromatin-remodeling complex are present in the yeast holoenzyme. Thus, one function of transcriptional activators is to recruit chromatin-remodeling machinery to specific promoters.

27. Gaudreau L, Schmid A, Blaschke D, Ptashne M, Horz W: **RNA polymerase II holoenzyme recruitment is sufficient to remodel chromatin at the yeast PHO5 promoter.** *Cell* 1997, **89**:63–72.

28. Lin YS, Carey M, Ptashne M, Green MR: **How different eukaryotic transcriptional activators can cooperate promiscuously.** *Nature* 1990, **345**:359–361.

29. Carey M, Lin YS, Green MR, Ptashne M: **A mechanism for synergistic activation of a mammalian gene by Gal4 derivatives.** *Nature* 1990, **345**:361–364.

30. • Chi T, Lieberman P, Ellwood K, Carey M: **A general mechanism for transcriptional synergy by eukaryotic activators.** *Nature* 1995, **377**:254–257.
This paper shows that the synergistic interactions of transcriptional activators *in vitro* correlate with the assembly of a TFIID–TFIIA complex on the promoter, thus supporting the view that activators recruit GTFs to the promoter and stabilize their interactions with DNA.

31. • Sauer F, Hansen SK, Tjian R: **Multiple TAFIIs directing synergistic activation of transcription.** *Science* 1995, **270**:1783–1787.
This paper shows that interactions between multiple activators and different TBP-associated factors (TAFs) can result in transcriptional synergy *in vitro*.

32. Giniger E, Ptashne M: **Cooperative DNA binding of the yeast transcriptional activator GAL4.** *Proc Natl Acad Sci USA* 1988, **85**:382–386.

33. Xiao H, Perisic O, Lis JT: **Cooperative binding of *Drosophila* heat shock factor to arrays of a conserved 5 bp unit.** *Cell* 1991, **64**:585–593.

34. Ohashi Y, Brickman JM, Furman E, Middleton B, Carey M: **Modulating the potency of an activator in a yeast *in vitro* transcription system.** *Mol Cell Biol* 1994, **14**:2731–2739.

35. Choy B, Green MR: **Eukaryotic activators function during multiple steps of preinitiation complex assembly.** *Nature* 1993, **366**:531–536.

36. Herschlag D, Johnson FB: **Synergism in transcriptional activation: a kinetic view.** *Genes Dev* 1993, **7**:173–179.

37. Krämer A: **The structure and function of proteins involved in mammalian pre-mRNA splicing.** *Annu Rev Biochem* 1996, **65**:367–409.

38. Reed R, Palandjian L: **Spliceosome assembly.** In *Eukaryotic mRNA Processing*. Edited by Krainer AR. Oxford, England: IRL Press; 1997:103–129.

39. Reed R, Maniatis T: **A role for exon sequences and splice site proximity in splice site selection.** *Cell* 1986, **46**:681–690.

40. • Reed R: **Initial splice-site recognition and pairing during pre-mRNA splicing.** *Curr Opin Genet Dev* 1996, **6**:215–220.
This minireview discusses a model for initial splice-site recognition that involves multiple weak interactions. In this model, a network of cross-exon protein–protein interactions bridges the splice sites on either side of the exon and recruits U2snRNP to the branchsite.

41. Watakabe A, Tanaka K, Shimura Y: **The role of exon sequences in splice site selection.** *Genes Dev* 1993, **7**:407–418.

42. Tian M, Maniatis T: **Positive control of pre-mRNA splicing *in vitro*.** *Science* 1992, **256**:237–240.

43. Chabot B: **Directing alternative splicing: cast and scenarios.** *Trends Genet* 1996, **12**:172–177.

44. Fu XD: **The superfamily of arginine/serine-rich splicing factors.** *RNA* 1995, **1**:663–680.

45. Manley JL, Tacke R: **SR proteins and splicing control.** *Genes Dev* 1996, **10**:1569–1579.

46. Wu JY, Maniatis T: **Specific interactions between proteins implicated in splice site selection and regulated alternative splicing.** *Cell* 1993, **75**:1061–1070.

47. Kohtz JD, Jamison SF, Will CL, Zuo P, Lührmann R, Garcia-Blanco M, Manley JL: **Protein–protein interactions and 5′-splice site recognition in mammalian mRNA precursors.** *Nature* 1994, **368**:119–124.

48. Valcarcel J, Singh R, Zamore PD, Green MR: **The protein Sex-lethal antagonizes the splicing factor U2AF to regulate alternative splicing of *transformer* pre-mRNA.** *Nature* 1993, **362**:171–175.

49. • Zuo P, Maniatis T: **The splicing factor U2AF^{35} mediates critical protein–protein interactions in constitutive and enhancer-dependent splicing.** *Genes Dev* 1996, **10**:1356–1368.
This paper provides evidence that the 35 kDa subunit of the essential splicing factor U2AF (U2AF^{35}) may function in cross-intron splice-site pairing in constitutive splicing, and as a link between the enhancer complex and the 3′ splice site in activated splicing. Thus, U2AF^{35} may be the primary target for recruitment by the enhancer complex.

50. Zahler AM, Roth MB: **Distinct functions of SR proteins in recruitment of U1 small nuclear ribonucleoprotein to alternative 5′ splice sites.** *Proc Natl Acad Sci USA* 1995, **92**:2642–2646.

51. • Chiara MD, Reed R: **A two-step mechanism for 5′ and 3′ splice site pairing.** *Nature* 1995, **375**:510–513.
RNAs can be spliced in *trans* if the 3′ splice site is associated with an exonic enhancer or a downstream 5′ splice site. As the selection of 5′ splice sites was shown to occur after U2snRNP is assembled on the 3′ splice-site, it is suggested that splice-site pairing can occur after initial splice-site recognition.

52. • Bruzik J, Maniatis T: **Enhancer dependent interaction between 5′ and 3′ splice sites in trans.** *Proc Natl Acad Sci USA* 1995, **92**:7056–7059.
Trans-splicing can occur if the 3′ substrate contains an exonic enhancer sequence in addition to the 3′ splice site signals. The *trans* reaction is mediated by SR proteins interacting with general splicing factors that are bound to the 5′ and 3′ splice sites.

53. Lynch KW, Maniatis T: **Synergistic interaction between two distinct elements of a regulated splicing enhancer.** *Genes Dev* 1995, **9**:284–293.

54. Tian H, Kole R: **Selection of novel exon recognition elements from a pool of random sequences.** *Mol Cell Biol* 1995, **15**:6291–6298.

55. • Tacke R, Manley JL: **The human splicing factors ASF/SF2 and SC35 possess different, functionally significant RNA binding specificities.** *EMBO J* 1995, **14**:3540–3551.
Using a binding site selection method, the authors identified consensus binding sites for the SR proteins ASF/SF2 and SC35. In the case of ASF/SF2, one of these binding sites could direct ASF/SF2-stimulated splicing *in vitro* when fused to a weak heterologous intron.

56. Tian M, Maniatis T: **A splicing enhancer exhibits both constitutive and regulated activities.** *Genes Dev* 1994, **8**:1703–1712.

57. Carey M, Leatherwood J, Ptashne M: **A potent GAL4 derivative activates transcription at a distance *in vitro*.** *Science* 1990, **247**:710–712.

58. •• Lynch KW, Maniatis T: **Assembly of specific SR protein complexes on distinct regulatory elements of the *Drosophila* doublesex splicing enhancer.** *Genes Dev* 1996, **10**:2089–2101.
Specific heterotrimeric complexes associate with each of the two classes of binding sites within the *dsx* enhancer. The formation of each of these complexes is dependent on highly cooperative interactions between the three proteins of the specific heterotrimeric complexes, and between the proteins and the RNA.

59. Tian M, Maniatis T: **A splicing enhancer complex controls alternative splicing of *doublesex* pre-mRNA.** *Cell* 1993, **74**:105–114.

60. Hedley ML, Maniatis T: **Sex-specific splicing and polyadenylation of *dsx* pre-mRNA requires a sequence that binds specifically to *tra-2* protein *in vitro*.** *Cell* 1991, **65**:579–586.

61. Ryner LC, Baker BS: **Regulation of doublesex pre-mRNA processing occurs by 3′-splice site activation.** *Genes Dev* 1991, **5**:2071–2085.

62. Hoshijima K, Inoue K, Higuchi I, Sakamoto H, Shimura Y: **Control of *doublesex* alternative splicing by *transformer* and *transformer-2* in *Drosophila*.** *Science* 1991, **252**:833–836.

63. • Min H, Chan RC, Black DL: **The generally expressed hnRNP F is involved in a neural-specific pre-mRNA splicing event.** *Genes Dev* 1995, **9**:2659–2671.
The authors of this paper identified an enhancer complex that binds to an intronic sequence and activates recognition of an upstream exon. Evidence is presented that this complex forms through cooperative interactions between a number of proteins.

64. Yeakley JM, Morfin JP, Rosenfeld MG, Fu XD: **A complex of nuclear proteins mediates SR protein binding to a purine-rich splicing enhancer.** *Proc Natl Acad Sci USA* 1996, **93**:7582–7587.

65. • Berget SM: **Exon recognition in vertebrate splicing.** *J Biol Chem* 1995, **270**:2411–2414.
This review summarizes the evidence for the role of cross-exon interactions in splice-site selection and exon definition.

66. Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG: **hnRNP proteins and the biogenesis of mRNA.** *Annu Rev Biochem* 1993, **62**:289–321.

67. McAfee JG, Soltaninassab SR, Lindsay ME, LeStourgeon WM: **Proteins C1 and C2 of heterogeneous nuclear ribonucleoprotein complexes bind RNA in a highly cooperative fashion: support for their contiguous deposition on pre-mRNA during transcription.** *Biochemistry* 1996, **35**:1212–1222.

68. Sebillon P, Beldjord C, Kaplan JC, Broody E, Marie J: **A T to G mutation in the polypyrimidine tract of the second intron of the human β-globin gene reduces *in vitro* splicing efficiency: evidence for an increased hnRNP C interaction.** *Nucleic Acids Res* 1995, **23**:3419–3425.