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REVIEW

Transcriptional activators and activation mechanisms

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ABSTRACT

Transcriptional activators are required to turn on the expression of genes in a eukaryotic cell. Activators bound to the enhancer can facilitate either the recruitment of RNA polymerase II to the promoter or its elongation. This article examines a few selected issues in understanding activator functions and activation mechanisms.

KEYWORDS activator, transcription, co-activator, enhancer, promoter, signal transduction, development

INTRODUCTION

Transcription is the process of copying (transcribing) the information from one strand of DNA into RNA by the enzyme called RNA polymerase (RNAP). In bacteria there is only one type of RNAP, but in eukaryotes there are three different types of RNAPs that transcribe different classes of genes (Hahn, 2004). RNAPII is responsible for transcribing protein-coding genes, whereas RNAPI and III are responsible for synthesizing rRNA and tRNA, respectively. Here we focus on transcription by RNAPII (referred to as RNAP from now on), which has been subject to intensive investigations (Kadonaga, 2004; Sims et al., 2004b; Malik and Roeder, 2010; Weake and Workman, 2010; Nechaev and Adelman, 2011). We will discuss mechanisms leading to increased levels of transcription, a process called activation.

In addition to the coding sequence, a typical class II gene contains at least two other types of DNA sequences that are required for initiating transcription. Promoters (also referred to as the core promoters), which are DNA sequences located upstream of the coding regions of the genes, help orient RNAP so that it "knows" where on DNA to start transcribing and in which direction. RNAP itself does not have the ability to recognize specific DNA sequences including the promoter sequences. Instead, a group of proteins, called general transcription factors (GTFs), help RNAP to find promoter sequences (Orphanides et al., 1996; Hampsey, 1998; Nechaev and Adelman, 2011). One of these GTFs is the TATA-box binding protein (TBP), which directly binds to the TATA sequence of the promoter. The protein complex assembled at the promoter is often referred to as the preinitiation complex or transcription machinery (or apparatus). This complex contains GTFs and RNAP. It also contains co-factors and chromatin modifying/remodeling factors that are part of the RNAP holoenzyme. Many of these additional factors play important roles in mediating transcription regulation by responding to regulatory proteins (Naar et al., 2001; Narlikar et al., 2002; Levine and Tjian, 2003; Malik and Roeder, 2000, 2010; Weake and Workman, 2010).

The second type of DNA elements required for initiating gene transcription is the regulatory elements, to which regulatory proteins bind. The elements that play positive roles in transcription are called upstream activation sequences (UASs) in yeast and enhancers in higher eukaryotes such as humans. These sequences (as well as the proximal-promoter elements) provide the binding sites for transcriptional activators that increase the levels of gene transcription. Genes in eukaryotic cells tend to stay inactive (off) unless they are specifically turned on by activators. This is obvious for genes that need to be turned on at precise times and locations in response to environmental or developmental signals. But this is also true for “housekeeping” genes that are transcribed ubiquitously; for these genes, their transcription is also dependent on the action of activators. Many enhancers are located upstream of the genes, but they have also been found in introns or even downstream of the genes (Blackwood and Kadonaga, 1998). Recent studies suggest

that, in addition to the primary enhancers, genes may also contain secondary, “shadow” enhancers (below). In addition, enhancers often contain binding sites for both activators and repressors to achieve the precise spatial and temporal patterns of gene transcription. Here we focus on activation mechanisms. We first discuss how a typical activator looks like and how it might activate transcription, followed by brief discussions of a few selected issues relevant to understanding activation mechanisms.

A TYPICAL TRANSCRIPTIONAL ACTIVATOR

A typical activator has two essential functions: DNA binding and transcriptional activation (Ptashne, 1988). The finding that these two functions of an activator are provided by two separable domains led to the suggestion that DNA binding *per se* was insufficient, though necessary, for activation (Brent, 2004; Brent and Ptashne, 1985; Keegan et al., 1986; Ptashne, 2004). According to our current understanding, the DNA binding domain of an activator provides the specificity for its action (in terms of which gene to activate), whereas its activation domain is responsible for stimulating transcription.

There are different families of DNA binding domains that form distinct three-dimensional (3-D) structures for DNA recognition (Garvie and Wolberger, 2001). These domains tend to bear names that depict their structural and/or functional properties or follow their founding members’ names. For example, a zinc-finger DNA binding domain uses zinc to maintain its 3-D structure required for DNA recognition. A basic region-leucine zipper (bZIP) domain contains a basic region (that contacts DNA) and a leucine zipper (that forms dimers). A homeodomain is a conserved 60-aa DNA binding domain initially identified in proteins encoded by *Drosophila* homeotic genes. A Rel homology domain is a DNA binding domain that bears the name of its founding member Rel. These DNA binding domains, and many others, recognize short, specific DNA sequences by making elaborate contacts with the bases in the major groove of the DNA double helix (Patikoglou and Burley, 1997; Garvie and Wolberger, 2001; Baird-Titus et al., 2006). Some, e.g., the high-mobility-group (HMG) domain, recognize DNA sequences by interacting with the minor groove (Travers, 2000) [The GTF TBP also binds to the TATA sequences by making contacts with the minor groove (Nikolov et al., 1992; Kim et al., 1993)]. Many activators can bind DNA sequences cooperatively with one another, which can increase the stability of the protein complexes formed at the enhancers (Adams and Workman, 1995; Ma et al., 1996).

Unlike DNA binding domains that require elaborate structures for DNA recognition, activation domains tend to be short protein sequences often with very limited sequence complexity (Hope and Struhl, 1986; Ma and Ptashne, 1987a, 1987b). There are different types of activation domains, which are named after their sequence characteristics, such as acidic, glutamine-rich, proline-rich, and alanine-rich. For the acidic class of activating sequences, it was estimated that 1% of the peptides encoded by random DNA sequences (from the *E. coli* genome) can activate transcription when fused to a DNA binding domain (Ma and Ptashne, 1987a). This finding further highlights the "relaxed" specificity between activation sequences and their target proteins (Ma, 2004), a feature that stands in contrast to the interaction mode between the DNA binding domains and their recognized DNA sites.

THE RECRUITMENT MODEL

What does an activator do to stimulate transcription? According to a well-established recruitment model, the ultimate goal of an activator bound at the enhancer is to bring the transcription machinery, in particular RNAP, to the promoter (Stargell and Struhl, 1996; Ptashne and Gann, 1997). Several lines of evidence suggest that recruitment is an important mechanism for transcriptional activation. First, for many genes, the GTFs and RNAP are absent from their promoters unless the genes are turned on by activators (Klein and Struhl, 1994; Chatterjee and Struhl, 1995; Li et al., 1999). Second, activation domains can interact with a wide array of target proteins, many of which are components of the transcription machinery, including the GTFs (e.g., TBP, TFIIB, TFIIE, and TAFs), co-factors and chromatin modifying/remodeling complexes (Ptashne and Gann, 1990; Orphanides et al., 1996; Malik and Roeder, 2000; Peterson and Workman, 2000; Naar et al., 2001; Narlikar et al., 2002). Finally, in a series of "artificial recruitment" experiments that provided pivotal support to this model, it was shown that transcription can be elicited by artificially attaching components of the transcription machinery to a DNA binding domain (Chatterjee and Struhl, 1995; Xiao et al., 1995; Farrell et al., 1996; Gonzalez-Gouto et al., 1997; Nevado et al., 1999). In such an artificial recruitment setup, the requirement for a classical activator is completely bypassed. Instead, “activation” is now achieved through a shortcut mechanism, where the transcription machinery is directly brought to its action site, the promoter, by a covalently-attached DNA binding domain. As discussed below, increasing evidence suggests that transcriptional activation for many genes may take place at a step(s) after recruitment.

COMPOSITE ACTIVATORS

Although a typical activator contains both an activation domain and a DNA binding domain, sometimes these two domains can reside on separate proteins. For example, the herpes simplex virus (HSV) activator VP16 does not bind to DNA, but rather, it is brought to DNA by interacting with other DNA-bound proteins (Triezenberg et al., 1988). The activation domain of VP16 can also activate transcription when directly

attached to a DNA binding domain (Sadowski et al., 1988). This finding demonstrated that an activation domain can be brought to DNA by distinct, but interchangeable, means, either directly binding to DNA (through its linked DNA binding domain) or interacting with other DNA-bound proteins. This concept was further demonstrated by the creation of an artificial composite activator (Ma and Ptashne, 1988) and led to the proposal of the yeast two-hybrid system (Fields and Song, 1989).

Transcriptional activators that do not themselves bind DNA but interact with other DNA-bound proteins are often referred to as co-activators. But it is useful to make a distinction between these non-DNA binding activators and the "true" co-activators that play more general roles in transcription (see below). Unlike non-DNA binding activators, which are gene-specific (e.g., the Notch intracellular domain), co-activators of the latter class (e.g., CBP and chromatin remodeling complexes) play important roles in mediating the actions of many activators (Ma, 2005). Some of these general, “true” co-activators are components of the RNAP holoenzyme (Ranish and Hahn, 1996; Myers and Kornberg, 2000).

CONFORMATIONAL CHANGES

The artificial recruitment experiments mentioned above support the notion that, for the genes tested, the ultimate and only function of activators is to bring RNAP to the promoter. It is known that the preinitiation complex undergoes several conformational changes before RNAP actually initiates transcription (Carey and Smale, 2000; Hirose and Ohkuma, 2007). For example, the promoter DNA is significantly bent and unwound upon TBP binding (Nikolov et al., 1992; Kim et al., 1993). In addition, the DNA double helix at the transcription start site becomes unpaired, or melted, to form a bubble prior to transcription initiation by RNAP (Wang et al., 1992; Giardina and Lis, 1993). In one study, it was shown that activators can change the conformation of the TFIIA-TFIID-TATA complex and such a conformational change is necessary and sufficient for activation in an *in vitro* system (Chi and Carey, 1996). Thus, conformational changes of the transcription machinery represent potential steps that can also be targeted by transcriptional activators.

PROMOTER CLEARANCE AND RELEASE OF PAUSED RNAP

The largest subunit of RNAP contains a tail-like structure known as the C-terminal domain (CTD), which is composed of heptapeptide repeats with a consensus of YSPTSPS (Meinhart et al., 2005). The number of heptapeptide repeats differs in different species; human RNAP has 52 such repeats whereas yeast RNAP has 26. The CTD, which is required for cell viability (Nonet et al., 1987), plays a critical role not only in transcription but also in RNA processing and chromatin regulation (Hirose and Ohkuma, 2007). Heptapeptide repeats are subject to extensive phosphorylation. Importantly, the phosphorylation status and specificity exhibit dynamic changes as a function of the transcription cycle, reflective of the balanced action of site-specific kinases and phosphatases (Meinhart et al., 2005). During preinitiation complex formation, the CTD is un-phosphorylated. It becomes phosphorylated at serine 5 (Ser5) when RNAP transitions from initiation to elongation, refereed to as promoter clearance or escape (Hirose and Ohkuma, 2007; Levine, 2011). This transition allows RNAP to gain competence to transcribe into the main body of the gene (Levine, 2011). Thus, promoter clearance represents an important step toward productive gene transcription. It has been suggested that Ser5 phosphorylation, catalyzed by the Cdk7 kinase in the TFIIH complex and the Cdk8 kinase in the mediator complex, may itself be responsible for this transition (Hirose and Ohkuma, 2007). As RNAP transcribes into the main body of the gene, its CTD becomes phosphorylated at serine 2 (see below).

It is well documented that, prior to the induction of some genes, RNAP is already engaged at their 5′ regions (after promoter clearance) but it becomes “stalled” or paused near the promoters (Rougvie and Lis, 1988; Krumm et al., 1992; Rasmussen and Lis, 1993, 1995; Levine, 2011). For these genes, which are referred to as having a “preloaded” RNAP, transcriptional activation must take place at a step(s) after RNAP recruitment and transcription initiation. Activation for these genes is thus a process of enabling the paused RNAP to elongate, i.e., to transcribe productively through the genes’ entire lengths. This process is referred to as the release of paused RNAP from the proximal promoter (Levine, 2011; Li and Gilmour, 2011; Nechaev and Adelman, 2011). Recent genome-wide studies have revealed that a significant fraction of genes have stalled RNAP near their promoters (Zeitlinger et al., 2007; Core et al., 2008; Nechaev et al., 2010). In addition, mutation of the polycomb group gene *extra sex combs* (*esc*) can cause an increased occupancy of RNAP at many genes, suggesting that the promoter type (with regard to the presence of preloaded RNAP) is sensitive to chromatin structure (Chopra et al., 2011). It has been suggested that promoters with preloaded RNAP can respond to activation signals more “quickly” and uniformly (Boettiger and Levine, 2009). In addition, paused RNAP has been suggested to provide a checkpoint to ensure a successful coupling between transcription and mRNA processing or to prevent “mistakes” during the transcription process (Sims et al., 2004a; Levine, 2011).

Many proteins (or complexes) have been identified that play important roles in facilitating transcription elongation, and some of these factors represent targets for activators (Sims et al., 2004a; Peterlin and Price, 2006). For example, experiments in *Drosophila* suggested that the elongation

factor P-TEFb is recruited to the heat shock loci to facilitate transcription elongation upon heat shock induction (Lis et al., 2000). The HIV Tat activator has also been suggested to stimulate transcription elongation by recruiting the elongation factor P-TEFb (Mancebo et al., 1997; Zhu et al., 1997; Zhou et al., 1998). P-TEFb is a protein complex that contains the Cdk9 kinase, which may directly phosphorylate Ser2 in the CTD thus facilitating the release of paused RNAP from the proximal promoter (Peterlin and Price, 2006; Levine, 2011). In addition, *in vitro* experiments using the human *hsp70* gene demonstrated that the SWI/SNF chromatin remodeling complex was recruited by the human activator HSF1 to facilitate transcription elongation (Brown et al., 1996). These examples highlight the importance of the elongation step in transcriptional activation (Levine, 2011; Li and Gilmour, 2011; Nechaev and Adelman, 2011).

CHROMATIN REMODELING AND MODIFICATIONS

A major difference between eukaryotes and prokaryotes is that eukaryotic DNA is packaged into nucleosomes (Li et al., 2007; Ho and Crabtree, 2010). Nucleosomes can impede DNA binding of transcription factors and GTFs and form a transcriptional barrier for RNAP. Thus, chromatin structure can significantly reduce the efficiency of transcription at both the initiation and elongation steps (Wu, 1997; Peterson and Workman, 2000; Wu and Grunstein, 2000; Narlikar et al., 2002; Li et al., 2007). It is well established that genes can be de-repressed when histones are depleted from cells (Han and Grunstein, 1988). A genome-wide analysis revealed that 15% of the yeast genes had a de-repressed (increased) expression upon the removal of histone H4 (Wyrick et al., 1999).

The role of chromatin structure in transcriptional activation has been subject to intensive mechanistic investigations (Li et al., 2007). These studies reveal two broad mechanisms that can lessen the repressive effects of chromatin structure. The first mechanism involves chromatin remodeling, a process where the contacts between histones and DNA are altered by ATP-dependent chromatin remodeling complexes (Li et al., 2007; Ho and Crabtree, 2010). These complexes are divided into four main families based on the sequence of the ATPase subunit, SWI/SNF, ISWI, CHD, and INO80 complexes (Ho and Crabtree, 2010). These complexes utilize ATP as energy to induce a variety of changes that increase the accessibility of nucleasomal DNA to transcription factors and GTFs and weaken the transcriptional barrier, thus leading to more efficient transcription initiation or elongation (Li et al., 2007; Ho and Crabtree, 2010).

The second mechanism involves posttranslational modifications of histone (Kouzarides, 2007; Li et al., 2007; Ruthenburg et al., 2007; Bannister and Kouzarides, 2011). Histones have flexible, unstructured N-terminal tails that are subject to a variety of posttranslational modifications, including acetylation, phosphorylation, methylation and ubiquitination (Kouzarides, 2007; Li et al., 2007; Ruthenburg et al., 2007; Bannister and Kouzarides, 2011). It is thought that many of these modifications, in particular acetylation, can reduce the net positive charge of nucleosomes, thus loosening their interactions with DNA and increasing the efficiency of transcription initiation or elongation (Li et al., 2007). Post-translational modifications of histones can also provide recognition platforms for other factors, referred to as the effector proteins, that further define the functional outcome of a modification in transcription (Li et al., 2007; Ruthenburg et al., 2007). It is noted that, since most of the chromatin remodeling complexes and histone modifying enzymes cannot bind specific DNA sequences, they are brought to the specific targets (genes) by transcriptional activators. They are thus referred to as transcriptional co-factors and, for those with a positive role in transcription, as co-activators (see above).

SYNERGISM

One of the characteristic features of transcriptional activation is synergism. Synergy refers to the situation where the transcription level achieved by multiple activators is higher than the sum of the levels by individual factors separately. Synergy can arise from different mechanisms. In the simplest case, it can be due to cooperative binding of activators to multiple sites in the enhancer (Adams and Workman, 1995; Ma et al., 1996; Burz et al., 1998). This is true if the activators are at limiting (sub-saturating) concentrations. An enhanceosome model has been proposed that further emphasizes the role of multiple activators for activation (Thanos and Maniatis, 1995; Merika and Thanos, 2001). According to this model, different activators, including those that play architectural roles, are together required to form a stable complex at the enhancer for efficient transcriptional activation. Synergy can also be achieved even when activators are at saturating levels, suggesting that activators may contact multiple targets in the transcription machinery (Ptashne and Gann, 1998).

Studies to compare the roles of different activators suggest that synergy may reflect combinatorial actions on distinct steps of transcription (Blau et al., 1996). By comparing the RNAP density along a gene, it is possible to gain information about which step, initiation or elongation, an activator may stimulate. Using this and other analyses, Blau et al. (Blau et al., 1996) concluded that, while some activators (e.g., Sp1 and CTF) work primarily on the initiation step, others (e.g., Tat) work primarily on the elongation step. Another class of activators (e.g., VP16, p53 and E2F1) can work on both initiation and elongation. An analysis of these activators revealed that synergy was only achieved between those that work on different steps of transcription (Blau et al., 1996).

ACTIVATOR-REPRESSOR SWITCHES

Transcription factors can often work as either activators or repressors in a context-dependent manner (Ma, 2005; Bauer et al., 2010). For example, many transcription factors that mediate signal transduction processes function as repressors in the absence of the signals but as activators in the presence of the signals. In addition, the concentrations and posttranslational modifications of a transcription factor can affect its ability to either activate or repress transcription. The presence of other nearby DNA binding proteins on DNA, as well as the availability and concentration of co-factors, can also influence the behavior of a transcription factor. Thus, understanding the precise role of a transcription factor in regulating the expression of a target gene requires the knowledge about the context in which it operates (Ma, 2005; Bauer et al., 2010).

ACTIVATOR MODIFICATIONS

Similar to histones, activators are also subject to a variety of posttranslational modifications, such as phosphorylation (Brivanlou and Darnell, 2002), acetylation (Brooks and Gu, 2003), and glycosylation (Jackson and Tjian, 1988; Kamemura and Hart, 2003). In many cases the posttranslational modifications can have positive roles in transcriptional activation. For example, phosphorylation of transducer and activator of transcription (STAT) is responsible for mediating the JAK/STAT signal transduction pathway (Darnell et al., 1994; Brivanlou and Darnell, 2002). Acetylation of p53 can increase its ability to bind DNA (Gu and Roeder, 1997; Prives and Manley, 2001; Brooks and Gu, 2003). Recent studies suggest that ubiquitination and sumoylation also play important roles in regulating the activity of transcription factors (Conaway et al., 2002; Herrera and Triezenberg, 2004; Gill, 2005; Ouyang and Gill, 2009; Frappier and Verrijzer, 2011). For many activators, their actions of stimulating transcription are coupled with their ubiquitination-dependent degradation (von der Lehr et al., 2003; Lipford et al., 2005; Muratani et al., 2005; Wu et al., 2007). Such a coupling, which was originally documented for classical activators that have activation domains, also appears to be operative for non-classical activators that do not have activation domains (Wang et al., 2010). Thus, cells have evolved mechanisms to maintain a “fresh” state by quickly eliminating the activator molecules that have fulfilled their duties of stimulating transcription.

SHADOW ENHANCERS

Recent studies suggest that, in addition to the primary enhancers, genes may also contain secondary enhancers, referred to as shadow enhancers (Hong et al., 2008). The primary enhancer and the shadow enhancer of a gene are often able to direct similar expression patterns. Thus these enhancers have redundant functions. It has been proposed that shadow enhancers can increase the robustness of gene expression (Frankel et al., 2010; Hobert, 2010; Perry et al., 2010). In addition, shadow enhancers may also have facilitated the creation of new regulatory DNA sequences through evolution (Hong et al., 2008).

SHORT DISTANCE *VS* LONG DISTANCE ACTIONS

Enhancers in higher eukaryotes have the ability to exert their effects even when they are located many kilobases away from the promoters (Blackwood and Kadonaga, 1998; Wallace and Felsenfeld, 2007; Ong and Corces, 2011). There are no clear-cut definitions of short distance *vs* long distance, but for our discussion we can consider short distance as anything up to a few hundred base pairs and long distance greater than one kilobase (Blackwood and Kadonaga, 1998; Dorsett, 1999). The mechanisms for activation at short or long distances may be fundamentally similar in that they are both achieved, ultimately, through a network of protein-protein interactions and alterations of chromatin structure. But activation at a long distance (e.g., 50–60 kilobases) faces two additional challenges that are less relevant to activation at a short distance (e.g., 100–200 bp). First, how can promoters and enhancers communicate through such long distances? Second, how does an enhancer "choose" to activate one promoter, but not another one that is also within its reach?

Proteins called facilitators have been proposed to promote the interaction between enhancers and promoters that are separated by long distances (Bulger and Groudine, 1999; Dorsett, 1999). For example, the *Drosophila* protein called Chip, which can interact with many transcription factors and co-factors (Morcillo et al., 1997; Torigoi et al., 2000; Matthews and Visvader, 2003; Bronstein et al., 2010), has been proposed to play such a role (Dorsett, 1999). In addition, recent studies have revealed critical roles of cohesin and non-coding RNAs in facilitating the communication between enhancers and promoters (Ong and Corces, 2011). The efficiency (and specificity) of the communication between enhancers and promoters can also be augmented by DNA sequences, called tethering elements, which are located near the core promoters (Bertolino and Singh, 2002; Calhoun et al., 2002). For example, the POU domain of Oct-1 bound to DNA sites near a promoter enables the promoter to respond to a distant enhancer (Bertolino and Singh, 2002). Finally, since eukaryotic genomes can have very large (on the scale of > 100 kilobases) domains that contain genes with similar expression patterns (Spellman and Rubin, 2002), it appears that some higher order control elements may play a role in controlling the genomic landscape of transcription (Calhoun and Levine, 2003; Spitz et al., 2003; Zuniga et al., 2004).

The specificity of long-distance communication between enhancers and promoters can be regulated by different

mechanisms (Blackwood and Kadonaga, 1998; Wallace and Felsenfeld, 2007). First, the tethering elements mentioned above can selectively facilitate the communication between a promoter and one, but not another, enhancer (Calhoun et al., 2002). Second, in some cases promoters can compete with each other for an enhancer, and thus the enhancer preferentially communicates with the strong promoter, while ignoring the weak promoter (Foley and Engel, 1992; Sharpe et al., 1998). Finally, insulator elements can prevent "unwanted" communications between enhancers and promoters thus encouraging "wanted" interactions; an insulator is a DNA element that can block the communication between an enhancer (or a silencer) and a promoter when the insulator is located between them, but not when it is located outside the enhancer-promoter unit (West et al., 2002; Kuhn and Geyer, 2003; Wallace and Felsenfeld, 2007).

TRANSCRIPTIONAL BURSTS

Our discussions thus far have intentionally avoided the question of what activation means in terms of the behavior of individual cells or individual copies of genes inside a cell. This is in part because most transcriptional studies measure the accumulated transcription products of many cells from a tissue. In other words, these measured products have already been averaged over many cells and, thus, depict only the “average” behavior of these cells as a group. Recent studies of evaluating the transcriptional products of individual cells suggest that transcription takes place as discrete bursts (Elowitz et al., 2002; Kaern et al., 2005; Raser and O'Shea, 2005; Golding and Cox, 2006; Pare et al., 2009; Chubb and Liverpool, 2010; To and Maheshri, 2010). Transcriptional activators appear to increase the frequency or probability of such bursts, as opposed to the number of transcripts produced per burst (Porcher et al., 2010; To and Maheshri, 2010; He et al., 2011).

Understanding mechanistically how activators work in their native biological systems as a function of both space and time represents an important scientific challenge. The *Drosophila* activator Bicoid (Bcd) forms a concentration gradient in early embryos and instructs anterior-posterior patterning (Grimm et al., 2010; Lohr et al., 2010; Porcher and Dostatni, 2010; Liu et al., 2011). It offers an excellent system for studying transcriptional activation mechanisms in both space and time (Gregor et al., 2007a, b; He et al., 2008; Deng et al., 2010; He et al., 2010a, b; Porcher et al., 2010; Cheung et al., 2011; Liu and Ma, 2011). A recently reported method of simultaneously, and quantitatively, detecting Bcd and the nascent transcripts of its target genes in early *Drosophila* embryos made it possible to evaluate the role of this activator in the actual events of transcriptional bursts, as opposed to the accumulated products (He et al., 2011). Our reported study captured for the first time the action of Bcd in stimulating the actual transcriptional bursts of individual copies of its target genes in a native developmental system. A comparison between the noise in transcriptional products and the noise in transcriptional events documented the effect of time averaging in reducing the noise in transcriptional output (He et al., 2011). Furthermore, a mathematical dissection of the measured noise in transcriptional bursts of Bcd target genes, coupled with the direct measurement of the Bcd activator input noise, has made it possible to evaluate—at a systems level—whether Bcd acts as a dominant input for its target gene transcription (He et al., in preparation). Finally, an analysis of a narrow time window for transcriptional bursts of a Bcd target gene has led to the demonstration that an important mechanism to control the levels of transcription products is to regulate the size of the transcriptional time window (Liu and Ma, in preparation).

CONCLUDING REMARKS

I would like to end our discussion by returning to the issue introduced earlier, i.e., a typical activator has two essential functions, DNA binding and activation. Why, then, do activators have to bind DNA, or for non-DNA binding activators, interact with other DNA-bound proteins? This question touches the very heart of the activation process. DNA binding brings an activator closer to the promoter, its action site, thus effectively increasing its local concentration. For an individual copy of a gene inside a cell, activator molecules bound at the enhancer can increase the probability of its transcriptional bursts; the stochastic nature of such bursts could reflect directly the stochastic binding of activator molecules to the enhancer or the stochastic (activator-facilitated) loading of RNAP to the promoter (Golding et al., 2005). For a promoter that is preloaded with RNAP, activator molecules bound at the enhancer can stimulate elongation of the stalled RNAP to achieve a productive transcriptional event. In both cases, activator molecules lead to successful transcriptional events only when they are brought closer to their action site. It should be noted that, while activation is a process of increasing transcription levels, transcription is also subject to repression, a process of reducing its levels. Understanding gene regulation requires considerations of integrating transcriptional activation and repression.

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ABBREVIATIONS

CTD, C-terminal domain; GTFs, general transcription factors; RNAP, RNA polymerase; TBP, TATA-box binding protein; UASs, upstream activation sequences

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