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The Enhanceosome and Transcriptional Synergy

Minireview

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Development of a complex eukaryote requires the differential transcription of over 50,000 genes in precise spatial and temporal patterns. One of the key problems in the gene expression field is understanding how an organism can achieve such diversity, while maintaining cell specificity and responding dynamically to its environment. One solution is to employ a limited repertoire of activators to minimize the complexity necessary to link related signaling pathways and to integrate diverse regulatory cues. The current view is that the cell accomplishes this by employing the principles of cooperativity and transcriptional synergy (Figure 1), where small combinations of ubiquitous, signal- and tissue-specific activators can be used to execute an exponentially larger number of regulatory decisions. Thus, an RNA polymerase II (pol II) enhancer responds to signals by organizing unique combinations of activators in a tightly clustered pattern that promotes their interaction and cooperative binding to DNA. The pol II transcriptional machinery, in turn, is designed to respond in a greater-than-additive or synergistic fashion only to multiple activators.

Previous studies from the Maniatis and Grosschedl laboratories on the IFNβ and TCRα gene enhancers, respectively, provided important biochemical details of how enhancer organization and cooperativity functioned to assemble activators into a nucleoprotein complex called the "enhanceosome." A key unanswered question was "how does the enhanceosome stimulate synergistic transcription and is the precise stereo-specific arrangement of activation domains necessary for the effect?" Recent biochemical studies reporting enhanceosome-activated transcription in vitro (Kim and Maniatis, 1997 [December issue of *Molecular Cell*]; Mayall et al., 1997) and the identification of activator "targets" within the transcriptional machinery (Bruhn et al., 1997; Merika et al., 1998 [January issue of *Molecular Cell*]) suggest that specific interaction surfaces are involved in synergy, and reveal new aspects of this regulation.

The Enhanceosome

The transition between the lysogenic and lytic states of bacteriophage λ in *E. coli* provided a paradigm for the role of cooperativity in gene regulation (Ptashne, 1992). Further studies established how cooperativity contributed to assembly of higher-order nucleoprotein structures, mediating what Echols termed "high-precision DNA transactions" during replication and site-specific recombination (Echols, 1986). These same principles were later invoked to describe the formation and function of enhanceosomes in eukaryotes (Giese et al., 1995; Grosschedl, 1995; Thanos and Maniatis, 1995).

Put simply, enhanceosome assembly is dependent on the arrangement of activator recognition sites and the precise complement of bound activators, which together generate a network of protein–protein and protein–DNA interactions unique to a given enhancer. The free energy of enhanceosome formation is fine-tuned to the concentration of the relevant activators in a cell and their ability to engage in combinatorial interactions; subthreshold concentrations (see Figure 1), the absence of key activators, or altered positioning on the DNA prohibit cooperative binding. As illustrated in Figure 2, the enhanceosome displays two layers of "stereo-specificity" necessary for gene activation. In one, the contextual activator–activator interactions promote cooperative assembly of the enhanceosome on naked DNA or chromatin templates, an issue addressed by several previous studies from the Maniatis and Grosschedl labs (Giese et al., 1995; Thanos and Maniatis, 1995; Kim and Maniatis, 1997) and a recent study by Jones and colleagues (Mayall et al., 1997). In the other, the enhanceosome displays a specific activation surface that is chemically and spatially complementary to "target" surfaces on coactivators and the basal pol II transcriptional machinery,

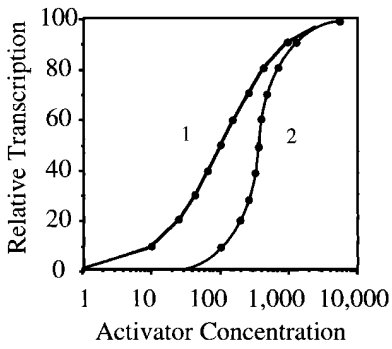

Figure 1. Manifestations of Synergy

There are two manifestations of synergy: The greater-than-additive transcriptional effect of multiple activator-binding sites on a promoter or enhancer, and the nonlinear or sigmoidal response of a gene to increasing activator concentrations. The vertical axis represents relative transcription. The horizontal axis denotes activator concentration (on a log scale). Curve 1 shows a standard parabolic response to increasing activator concentration for a reaction in which activators bind DNA noncooperatively and only a single activator is necessary to recruit the general machinery. Curve 2 shows the sigmoidal shape achieved by cooperative activator binding and by imposing a requirement for multiple activators, either the same molecule or combinations, to recruit the transcriptional machinery. The basis of the synergistic effect lies in a simple extrapolation of the Gibbs free energy equation, where the affinity of protein–protein interactions is exponentially related to the energy ($K = e^{\Delta G/-RT}$). The steepness of curve 2 would be influenced by the reciprocal cooperative effects of the transcriptional machinery and the activators. The curves are normalized to occupancy of 90%, or near maximal transcription. The enhanced sensitivity imparted by cooperativity is emphasized by the smaller difference in activator concentration required to increase relative transcription from 10% to 90% in curve 2 versus curve 1. Note that activators need not bind cooperatively to DNA for combinatorial control to function at a fundamental level although multiple interactions with the general machinery are essential.

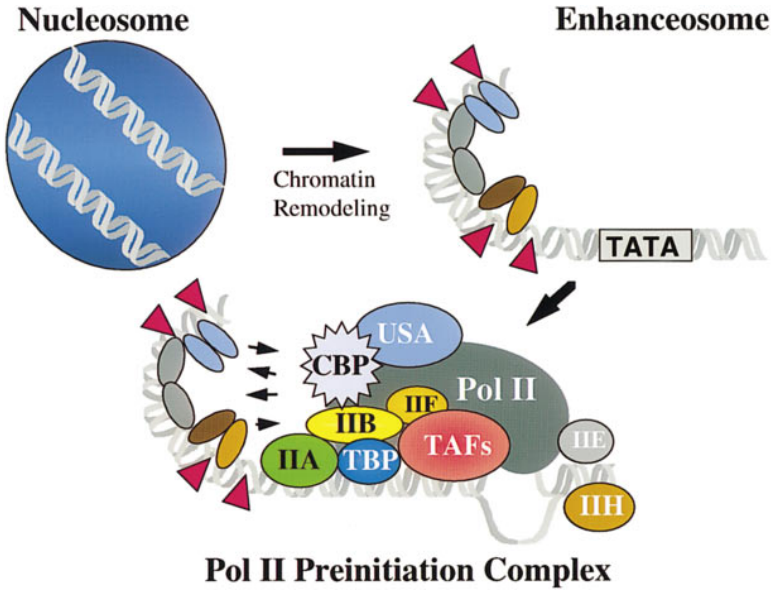

Figure 2. Cooperativity Functions in Enhanceosome and Preinitiation Complex Assembly

The final transcription complex assembles in a multistage process, each step of which is cooperative. Initially, activators bind to chromatin in a manner that is inherently cooperative (Kingston et al., 1996). Multiple sequence-specific activators (ovals) and DNA-bending proteins (triangles) then engage in cooperative protein–protein interactions to form a stable enhanceosome. The enhanceosome presents a distinct surface complementary to a surface displayed by coactivators and the pol II general machinery. This leads to cooperative recruitment of pol II and its ancillary factors to DNA and results in synergistic transcription. The reverse and forward arrows indicate reciprocity in the interactions. Although the process is delineated into separate steps, the reciprocity may drive concerted assembly of a transcriptosome.

recruiting them to DNA to generate synergistic transcription (Figure 2), an issue addressed in three recent papers (Bruhn et al., 1997; Kim and Maniatis, 1997; Merika et al., 1998). Because enhanceosomes form on short segments of DNA (~100 bp or less), their assembly is facilitated by "architectural" proteins that allow protein–protein interactions normally proscribed by the energetic cost of DNA bending and twisting within its persistence length (Grosschedl, 1995). An important theme throughout the entire process is reciprocity—the enhanceosome recruits the pol II machinery, but the machinery reciprocally facilitates assembly of the enhanceosome. This latter effect could, in principle, provide the additional specificity and energy necessary to drive the concerted formation of the final "transcriptosome" in the face of the large energetic obstacle posed by chromatin (Kingston et al., 1996).

Enhanceosomes that embody the aforementioned principles have now been identified and characterized biochemically on several model enhancers in both *Drosophila* (Courey and Huang, 1995) and mammalian systems. Figure 3 illustrates the prototypic cases: TCRα and IFNβ (Giese et al., 1995; Kim and Maniatis, 1997; Mayall et al., 1997; Merika et al., 1997). The p50 and p65 subunits of NF-κB, IRF-1, ATF-2, c-Jun, and HMG I bind IFNβ (Figure 3), while the sequence-specific regulatory proteins Ets-1, AML-1 (CBFα2, PEB2αB), LEF-1, and ATF (or CREB) bind TCRα.

Although many of the factors constituting the enhanceosome are traditional gene activators, LEF-1 and HMG I are sequence-specific DNA-bending proteins from two distinct classes of chromatin-associated high-mobility group (HMG) proteins. LEF-1 (Lymphoid enhancer factor 1) contains a conserved 79-amino acid HMG domain, also found in the ubiquitous HMG-1 and −2 proteins, which binds in the minor groove and intercalates a hydrophobic amino acid between adjacent base pairs in the site. The HMG domain bends and untwists the DNA, molding the minor groove to fit the contour of the protein (Grosschedl, 1995), much like TBP at the TATA box (Roeder, 1996). The resulting 120° bend permits cooperative interactions between ATF (or possibly CREB), AML-1, and Ets-1. The 107-amino acid HMG I protein (also called HMG I/Y and not to be confused with HMG-1) contains three repeated basic DNA-binding domains separated by short linkers. At least two of the domains simultaneously interact with the minor groove of different AT-rich sequences in the enhancer (Yie et al., 1997). HMG I does not dramatically alter DNA shape or minor groove trajectory. The current idea is that the multivalent interactions form a clamp, which reverses a mild, yet inhibitory, 20° DNA bend toward the minor groove, allowing NF-κB to bind its site. Unlike LEF-1, which contains a context-dependent activation domain, HMG I does not participate directly in stimulation but does facilitate cooperative assembly of the enhanceosome (Kim and Maniatis, 1997). Note that studies in several systems have shown that architectural proteins can be bypassed if the strength or flexibility of the interactions can absorb the energetic cost of DNA distortion.

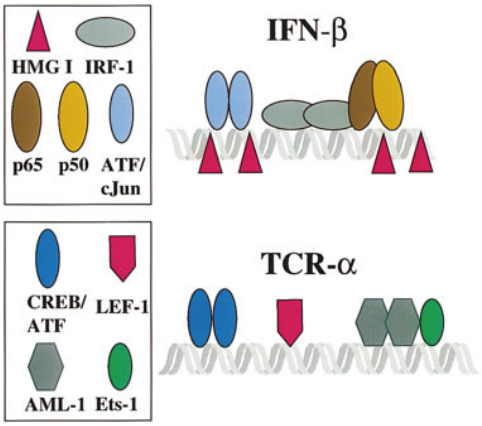

Figure 3. Two Prototypic Enhanceosomes

A schematic of the 75 bp TCRα and 57 bp IFNβ enhanceosomes.

Further, the bending need not be sequence specific as shown with the relatively nonspecific HMG-1 yeast homologs NHP6A and 6B (Paull et al., 1996).

Specificity in Enhanceosome Assembly

In the case of the IFNβ, artificial promoters bearing tandem copies of each activator-binding site responded moderately to a range of extracellular signals. However, the individual sites responded only to viral induction when they were organized in a specific context within the enhanceosome. Several of the activators are modified by kinase-dependent signaling systems, and it is believed that phosphorylation increases their affinities for each other and for coactivators (Karin et al., 1997; Montminy, 1997). The simultaneous, albeit modest increase in the affinities of multiple interacting components could, in principle, lead to cooperative DNA binding and explain the highly synergistic transcriptional response observed in vivo (see Figure 1 legend).

In their most recent study, Kim and Maniatis (1997), using an IFNβ enhanceosome assembled in vitro, were able to reproduce synergistic transcriptional activation and elucidate its underlying regulatory mechanism. After depleting mammalian nuclear extracts of endogenous IFNβ-binding proteins by DNA- and immunoaffinity chromatography, the authors supplemented the extract with limiting concentrations of the individual recombinant proteins and recapitulated the highly synergistic response observed in vivo. The ability to manipulate activator concentration allowed the authors to test whether the synergism could be obtained under conditions in which the template sites were occupied. While saturating concentrations of NF-κB, IRF-1, ATF-2, and c-Jun could circumvent the requirement for HMG I, the absence or repositioning of any other factor abolished the synergy. This result implies that the cooperativity not only facilitates binding of the activators to DNA but also positions them to create a stereo-specific interface for docking with and recruitment of the transcriptional machinery.

Recruitment of the Pol II Transcription Machinery

The general transcription machinery in eukaryotes, in addition to pol II comprises six general factors called TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH and a series of coactivators that allow transcriptional stimulation above basal levels (Figure 2). The TAF subunits of TFIID, the USA coactivator fraction, and CBP are all coactivators, which increase gene activation in vitro or in vivo (Roeder, 1996; Montminy, 1997).

Previous studies established that multiple interactions between activators and the general machinery can lead to synergistic transcription. For example, multiple molecules of bound GAL4-VP16 or the EBV transactivator ZEBRA activate transcription synergistically. Remarkably, the synergy is most apparent with only two or three upstream activators, suggesting that recruitment of a limited set of general factors is sufficient for the effect. In support of this idea, both activators cooperatively recruit subcomplexes containing only TFIID, TFIIA, and TFIIB to a nearby core promoter (Chi et al., 1995).

The notion that contacting a limited repertoire of targets is sufficient for activation is further supported by studies in yeast. Tethering a single fusion protein, bearing a transcriptionally inert DNA-binding domain (i.e., LexA) linked to any one of several different components of the general machinery (i.e., TBP, TFIIB, TAFs, or GAL11) generated activated levels of transcription on model promoters (Ptashne and Gann, 1997). These data imply that individual general factors, when recruited to a promoter, have the capacity to nucleate assembly of a functional transcription complex around themselves. Therefore, although the total mass of the complex in mammalian cells has been estimated to exceed 2.5 MDa and contain dozens of polypeptides, activators need interact with only a small portion of the overall surface, or a few targets within it, to stimulate transcription (Ptashne and Gann, 1997).

While these studies were performed in unregulated, model systems, they form the foundation for added specificity in combinatorial control. Different surfaces on the general machinery could be designed to interact with distinct spatial arrangements of activation domains. Furthermore, because different factors in the complex play unique regulatory roles, the "target" surface could vary depending upon the regulatory context. The coactivator CBP, for example, interacts at multiple points along a contiguous surface, with each of the activators in the IFNβ enhanceosome (Merika et al., 1998). Removal of the activation domains, replacement with VP16, or altered helical phasing of the sites, abolishes CBP-dependent synergistic activation in vivo and efficient recruitment to the enhanceosome in vitro. Deletion analysis showed that the p65 subunit of NF-κB contains both a general activation domain, possibly for interaction with the general factors, and a "synergy domain" necessary for interaction and recruitment of CBP. Removal of the synergy domain severely reduced the ability of NF-κB to activate in the context of the enhanceosome. Studies by Jones and colleagues implied a requirement for phosphorylated CREB, and thus CBP, in transcription from TCRα enhanceosomes formed on chromatin (Mayall et al., 1997). Additionally, Grosschedl and colleagues have shown that a different coactivator called ALY apparently interacts specifically with the combination of LEF-1 and AML-1 (Bruhn et al., 1997).

The enhanceosome also interacts with general transcription factors and coactivators such as USA. In vitro transcription experiments by Kim and Maniatis (1997) demonstrated that when the enhanceosome was preincubated with TFIIA, TFIIB, TFIID, and USA, a transcription complex resistant to the detergent sarkosyl was formed. Taken together, these data suggest that the enhanceosome recruits both coactivators and the general factors, which collectively add to the stability of the final complex. It is conceivable that some of these identified targets are assembled in the form of a holoenzyme and that the recruitment steps are mechanistically linked in vivo (Ptashne and Gann, 1997).

Reciprocity—an Additional Layer of Specificity

The idea that the enhanceosome engages in multiple, specific contacts with coactivators and the general machinery predicts that those interactions will reciprocally stabilize the assembly of the enhanceosome. Indeed, Kim and Maniatis (1997) showed that the complex of TFIIA, TFIIB, TFIID, and the USA coactivators had a reciprocal effect on enhanceosome stability, enabling it to survive challenge by competitor oligonucleotides.

The TFIIE, TFIIF, TFIIH, and pol II fractions, which apparently contain some CBP, further increase the stability. A more direct reciprocal effect was observed with the coactivator ALY, which dramatically enhanced cooperative binding of LEF-1 and AML-1 to TCRα in footprinting experiments (Bruhn et al., 1997). Though the issue was not addressed in these experiments, other studies have shown that the magnitude of the reciprocity is dependent upon the strengths of the activator–target interactions (see Tanaka, 1996).

New Directions

While significant progress on understanding the assembly and function of enhanceosomes has been made over the past year, several important issues remain unresolved. In the case of IFNβ, for example, because the enhanceosome assembles in vitro from unmodified recombinant proteins, what is the role of signal-dependent, posttranslational activator modifications necessary for enhanceosome function in vivo (e.g., phosphorylation)? Additionally, Kim and Maniatis (1997) point out that genetic disruption of IRF-1 does not affect viral induction of IFNβ: Is IRF-1 assembled into an enhanceosome in vivo or does a closely related family member take its place?

The notion of "stereo-specificity" in activator–target docking needs to be further clarified. For example, unlike the case of LexA-TBP and similar fusions in yeast (Ptashne and Gann, 1997), tethering CBP or ALY to a promoter does not bypass the requirement for the activators (Bruhn et al., 1997; Merika et al., 1998). Must activators, coactivators, and the general factors all interact in mammalian cells to generate synergistic activation? Additionally, the IFNβ enhanceosome functions in both orientations, even when positioned close to the core promoter. This observation apparently contradicts the strict idea of stereo specificity established in studies on site-specific recombination complexes.

Finally, in closing, there are tremendous differences in the range and action of enhancers. The ideas and studies presented here describe how specificity can be achieved. It is apparent, however, that the broadly active SV40 enhancer, with its built-in redundancy in activator-binding sites (Ondek et al., 1988), the cell-specific TCRα enhancer tuned to the activators present in a particular cellular milieu, and the signal-dependent IFNβ enhancer have common and distinct properties. A comparison of the biochemical details of enhanceosome formation in different contexts will reveal how its dynamics and stability can be adjusted to accommodate alternate regulatory scenarios. Examination of the roles played by LEF-1 and HMG I in different enhanceosomes will determine whether their functions can be generalized or are context specific (see Yie et al., 1997, and references therein).

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