REVIEW: TRANSCRIPTION

Transcriptional Coregulators in Development

Mattias Mannervik, Yutaka Nibu, Hailan Zhang, Michael Levine*

Small differences in the levels of an extracellular signaling molecule can specify cell fate during development. Threshold responses are often determined at the level of transcription. Cell-specific and spatially localized patterns of gene expression depend on combinations of sequence-specific activators and repressors that bind to extensive cis-regulatory regions. Different mechanisms for integrating this complex regulatory information are discussed, particularly the role of coregulatory proteins, which are recruited to the DNA template by sequence-specific transcription factors. Recent studies suggest that a growing set of coactivators and corepressors mediate communication between diverse upstream regulatory proteins and the core RNA polymerase II transcription complex.

The focus of this review is on the role of coactivators and corepressors in the regulation of gene expression during development, with particular reference to the *Drosophila* embryo. There is mounting evidence that such proteins link sequence-specific activators and repressors to the core RNA polymerase II (Pol II) complex. The first part of the review summarizes the activities of the CBP (CREB binding protein) histone acetyltransferase, a general activator that has been implicated in chromatin decondensation (*1*, *2*). The second part examines several corepressor proteins, including Groucho (*3*), CtBP (COOH-terminal binding protein) (*4*–*7*), and the Rpd3 (reduced phosphate dependency) histone deacetylase (*2*, *8*).

CBP and Transcriptional Activation

The RNA Pol II complex is composed of about 40 different subunits. Early studies suggested that a number of these proteins, the general transcription factors (GTFs) TFIIA, TFIIB, and TATA binding protein (TBP), as well as several different TBP-associated factors (TAF_{II}s), can serve as direct targets for sequence-specific activators (*9*). Recent studies suggest that coactivators, and coactivator complexes, can also link upstream activators to the core Pol II complex (Fig. 1).

CBP is the most widely characterized coactivator protein (*1*). It was first shown to interact with the bZIP (basic leucine zipper) transcription factor CREB (cylic AMP response element binding protein) (*10*). More recent studies have implicated CBP and the related p300 protein (*11*) as essential coactivators of a variety of transcriptional activators, including the retinoic acid receptor (RAR) and thyroid hormone receptor (TR), and two Rel-containing transcription factors, the p65 subunit of nuclear factor (NF) κB, and Dorsal [(*12*–*14*); see (*1*, *12*, *15*) for additional examples of CBP-dependent transcriptional activators].

CBP possesses a histone acetyltransferase activity that is thought to decondense chromatin and facilitate the binding of the Pol II transcription complex to the core promoter (*16*). Recent studies suggest that CBP is a component of large protein complexes containing additional histone acetyltransferases, including p/CAF (p300/CBP associated factor) and steroid receptor coactivators (SRCs) (*12*). Critical CBP-dependent activator sites can be located over 1 kb from the transcription start site (*17*), and it is difficult to imagine how CBP-containing complexes can decondense the core promoter over such long distances. CBP also associates with other coactivator complexes, such as ARC (activator-recruited cofactor) (*18*). ARC contains mammalian homologs of yeast mediator proteins (*19*) that interact with Pol II and somehow facilitate transcription (*20*). Thus, it would appear that CBP functions in concert with coactivators that directly interact with Pol II (Fig. 1). CBP itself might have a dual role in chromatin decondensation and Pol II recruitment because it has been shown to interact with RNA helicase A, a subunit of a large Pol II complex (*21*). Similarly, the yeast SAGA coactivator complex (*22*) contains both a histone acetyltransferse (GCN5) and general transcription factors, such as TAF_{II}s, which are components of the Pol II complex.

Genetic studies in *Drosophila* (*14*, *23*) and *Caenorhabditis elegans* (*24*) indicate an essential role for CBP in development. CBP has been proposed to interact with the Dorsal transcription factor in the early *Drosophila* embryo (*14*). Dorsal is initially distributed throughout the cytoplasm of mature eggs, but shortly after fertilization it is released into nuclei. At the time of this nuclear transport process the embryo is a syncytium, and most of the nuclei are distributed along the periphery of the egg and enclose the internal yolk. Dorsal enters nuclei located in ventral regions of the embryo, whereas protein present in dorsal regions remains in the cytoplasm; small amounts of protein enter nuclei in lateral regions. This gradient establishes different thresholds of gene activity and tissue differentiation by regulating target genes in a concentration-dependent manner (*25*) (Fig. 2). Although interactions between Dorsal and two TAF_{II}s, TAF_{II}60 and

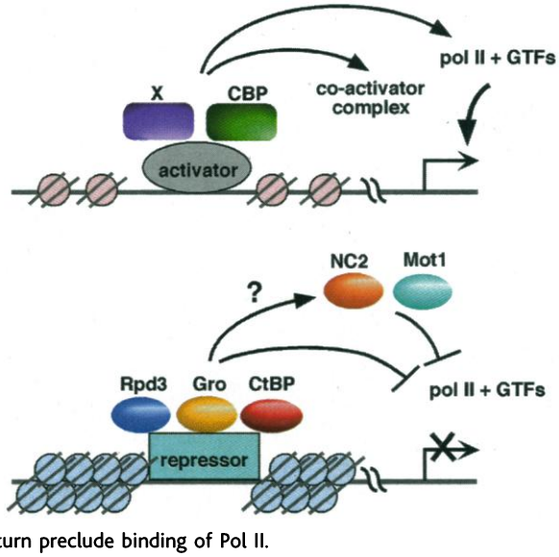

Fig. 1. Multiple tiers of activation and repression. **(Top)** Sequence-specific activators bound to a distal enhancer recruit the CBP coactivator. Recent studies suggest that CBP is a subunit of larger coactivator complexes. These complexes might mediate activation through the decondensation of chromatin at the core promoter, and by interacting with either the Pol II complex or general coactivator complexes, which in turn facilitate the binding of the general transcription factors (GTFs) and Pol II. **(Bottom)** Similarly, distal repressors recruit corepressor complexes that might also possess multiple activities. Perhaps some subunits, such as Rpd3, condense chromatin, whereas other subunits interact with general repressor complexes (NC2, Mot1), which in turn preclude binding of Pol II.

Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed. E-mail: mlevine@uclink4.berkeley.edu

$TAF_{II}110$, contribute to Dorsal-mediated activation (*26*), CBP might also function as a coactivator of Dorsal (*14*). Mutations in the *Drosophila* homolog of CBP, *nejire*, can result in a failure to activate the Dorsal target gene *twist*, which is essential for mesoderm differentiation.

Mutations in the *C. elegans* homolog of CBP (CBP-1) cause severe disruptions in the differentiation of several embryonic tissues, including the gut and muscles (*24*). Many of these defects are at least partially suppressed by eliminating the activity of the *C. elegans* homolog of the yeast Rpd3 histone deacetylase. The fact that CBP-1; Rpd3 double mutants are nearly normal suggests that most transcription factors are able to activate transcription in the absence of CBP when chromatin-condensing enzymes such as histone deacetylases are inactivated. This raises the possibility that there is another tier of transcriptional activation, which involves direct contact with the Pol II complex, as discussed above.

Corepressors and Transcriptional Repression

As in the case of activation, early studies on repression suggested that sequence-specific repressors can make direct contact with different components of the Pol II transcription complex, particularly TBP and the β subunit of TFIIE (*27*). More recent studies have identified the Rpd3 histone deacetylase as a critical corepressor of several mammalian regulatory proteins, including the basic-helix-loop-helix (bHLH) Mad/Max heterodimer, and the RAR and TR nuclear receptors (*12*). As for CBP, Rpd3 appears to be a subunit of a larger protein complex, one that includes Sin3 (SWI-independent), SMRT (silencing mediator for retinoid and thyroid-hormone receptors), and N-CoR (nuclear receptor corepressor), among other proteins (*12*). These latter proteins are thought to function as adapters that allow the Rpd3 corepressor complex to interact with a diverse array of sequence-specific repressors. Rpd3 might mediate repression by condensing chromatin at the core promoter or distal enhancer, and thereby impede the binding of upstream activators or core components of the Pol II transcription complex (Fig. 1). Although CBP has been identified as a coactivator for a number of unrelated sequence-specific activators, it is currently unclear whether Rpd3 and other histone deacetylases are equally pervasive as corepressors. In fact, preliminary genetic studies suggest that the Rpd3 homolog in *Drosophila* is not essential for the activities of most of the sequence-specific repressors present in the early embryo (*28*). However, a recent study raises the possibility that the Hunchback repressor interacts with a homolog of the Mi-2 protein (*29*), which is a member of the Snf2 family of chromatin-remodeling ATPases (adenosine triphosphatases) and is contained within a protein complex that includes the Rpd3 histone deacetylase (*30*).

At least 10 different sequence-specific repressors help establish localized stripes, bands, and tissue-specific patterns of gene expression in the syncytial *Drosophila* embryo (*31*). These repressors appear to fall into two categories, short-range and long-range (*32*). Short-range repressors work over distances of less than 100 base pairs to inhibit the core promoter or quench upstream activators. In contrast, long-range repressors can work over distances of 1 kb or more to silence transcription. Short-range repression represents a flexible form of gene regulation that can account for how different enhancers work independently of one another in a common promoter region. A short-range repressor bound to one enhancer does not interfere with activators in a neighboring enhancer. Recent studies suggest that these two modes of repression might depend on

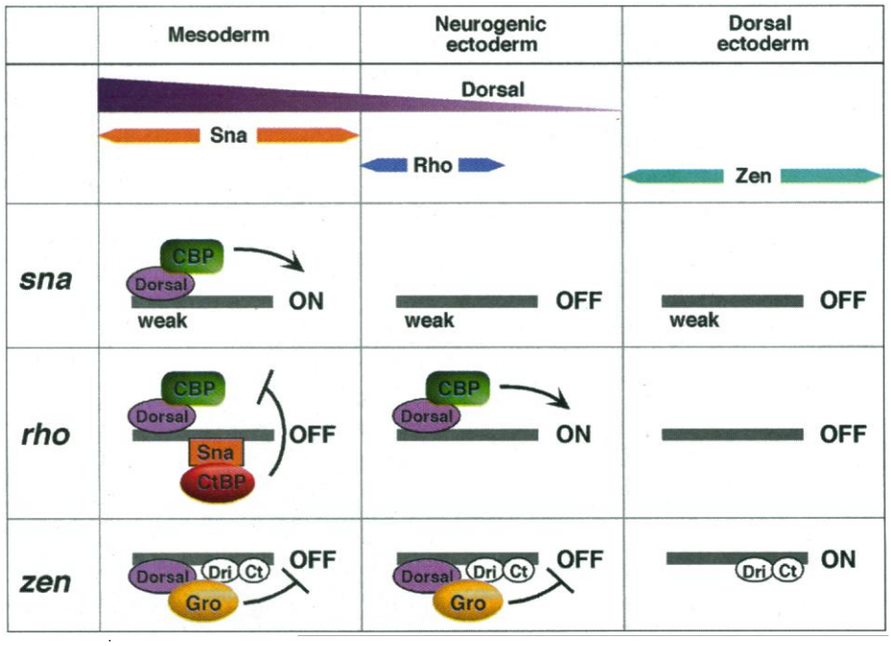

Fig. 2. Summary of Dorsal gradient thresholds. The Dorsal protein (depicted in purple at top) is distributed in a broad nuclear gradient with peak levels in the ventral mesoderm and progressively lower levels in the neurogenic ectoderm and dorsal ectoderm (drawn left to right). This gradient leads to differential patterns of *snail* (*sna*), *rhomboid* (*rho*), and *zerknüllt* (*zen*) expression, as indicated by the orange, blue, and aquamarine bars near the top of the diagram. The promoter regions of *twist* (*twi*) and *sna* contain weak Dorsal binding sites that interact with only high levels of the gradient, thereby restricting their expression to the mesoderm. Only the *sna* promoter region is shown. Beginning with "*sna*" in the leftmost column, the diagram shows the occupancy of the *sna* promoter region in the mesoderm, neurogenic ectoderm, and dorsal ectoderm. In this diagram, "CBP?" refers to the possibility that Dorsal-mediated activation depends on a coactivator complex that contains CBP. We note, however, that neither *sna* nor *rho* has shown to be dependent on CBP. Dorsal-CBP activator complexes might form only in the mesoderm owing to low-affinity Dorsal operator sites and limiting amounts of the Dorsal protein. There are insufficient levels of Dorsal to bind the *sna* promoter region in the neurogenic ectoderm and dorsal ectoderm, so the gene is off in these regions. The *rho* target gene contains optimal Dorsal binding sites that can mediate activation by both high and low levels of the Dorsal gradient in the ventral mesoderm and lateral neurogenic ectoderm. However, the Sna protein functions as a repressor to keep *rho* off in the mesoderm. Sna interacts with the CtBP corepressor, so the diagram depicts the *rho* enhancer as containing both Dorsal-coactivator complexes and Sna-CtBP repressor complexes in the mesoderm. CtBP might inhibit the coactivator within the limits of the *rho* enhancer or, alternatively, it might block the binding or function of the Pol II complex at the core promoter. *rho* is expressed in the neurogenic ectoderm owing to the absence of the Sna repressor and the presence of sufficient levels of Dorsal to bind the *rho* enhancer. It is conceivable, but not yet known, that Dorsal recruits the CBP coactivator to the *rho* enhancer, as drawn in the diagram. Finally, *rho* is not expressed in the dorsal ectoderm owing to insufficient levels of Dorsal. *zen* is kept off in both the mesoderm and neurogenic ectoderm by the Dorsal gradient. In this case, Dorsal interacts with two sequence-specific regulatory proteins, Cut (Ct) and Dri within the *zen* silencer element, and the resulting complex recruits the Groucho (Gro) corepressor. *zen* is thought to be activated by ubiquitous factors in the early embryo, and expression is restricted to the dorsal ectoderm, where there is no Dorsal protein and no Dorsal-Cut-Dri repressor complexes in the *zen* promoter region.

two different corepressor proteins, Groucho and dCtBP.

Groucho was initially identified as a corepressor of Hairy (*33*), a bHLH repressor that is essential for segmentation and neurogenesis (*34*). The COOH-terminus of Hairy contains a specific amino acid sequence motif, WRPW (*35*), that is essential for interaction with Groucho (*3*). A variant of this motif, WRPY, is conserved in another pair-rule transcription factor, Runt (*36*). Moreover, several repressors lacking the WRPW or WRPY motif can also interact with Groucho, including Dorsal and Engrailed (*37*). In total, as many as half of the best characterized repressors present in the early embryo interact with Groucho (*33*, *36*, *37*). Two of these, Hairy and Dorsal, have been tested with regard to range of action, and both function as long-range repressors (*38*). Groucho is related to the Tup1 corepressor in yeast, which is thought to position nucleosomes over the core promoter or directly inhibit the Pol II complex (*39*).

Genetic studies have identified a second corepressor in the *Drosophila* embryo, dCtBP (*5*–*7*), a homolog of the human CtBP protein that was first identified on the basis of binding to the COOH-terminal region of the adenovirus E1A protein (*4*, *40*). CtBP binds to a specific sequence motif in E1A, PXDLSXK. This motif is also conserved in three repressors present in the early embryo, Snail, Knirps, and Krüppel. Mutant embryos that lack maternal dCtBP products exhibit patterning defects that can be attributed to the loss of Snail, Knirps, and Krüppel activity (*6*). All three repressors function over short distances, thereby raising the possibility that the dCtBP corepressor mediates short-range repression. Both dCtBP and mammalian CtBP proteins are related to 2-hydroxy acid dehydrogenases, so it is conceivable that they mediate repression through the enzymatic modification of chromatin (*4*, *5*, *7*, *41*). Regardless of mechanism, it currently appears that there are two modes of repression, short-range and long-range, which depend on two different corepressors, dCtBP and Groucho, respectively. Future studies will determine whether Groucho or dCtBP interact with histone deacetylases (*42*) or with general repressor complexes, such as NC2, Mot1, or Not, which interfere with the binding or assembly of the Pol II complex (*43*) (Fig. 1).

Transcription Factors Can Interact with Multiple Coregulators

There are several examples of transcription factors that interact with multiple coregulators. The adenovirus E1A protein represents one of the most thoroughly investigated cases (*44*). E1A has been shown to interact with three different coregulators: CBP, CtBP, and the retinoblastoma protein (Rb); the latter two proteins mediate repression. Similarly, a variety of studies suggest that Dorsal is an activator, but in specific cis-regulatory regions it associates with two DNA binding proteins, Cut and Dead ringer (Dri), and is converted into a potent repressor through the recruitment of Groucho (*45*) (Fig. 2). Thus, Dorsal appears to interact with both coactivators and the Groucho corepressor. These interactions depend on conserved sequences in the Rel homology domain of Dorsal, which raises the possibility that mammalian Rel proteins, such as NF-κB, may also function as both activators and repressors. Furthermore, the *Drosophila* Hairy protein contains two different repression motifs: WRPW, which interacts with Groucho, and PXSLVXK, which weakly interacts with dCtBP (*7*, *33*). As discussed earlier, Hairy is engaged in a variety of processes including segmentation and neurogenesis (*34*). Perhaps some of these diverse activities depend on the differential recruitment of Groucho versus dCtBP (*46*).

The ability of a given sequence-specific transcription factor to interact with both coactivators and corepressors might provide a simple means for generating on-off switches in development. The *Drosophila* homolog of the mammalian TCF (T cell factor) regulatory factor, dTCF/Pangolin, provides a good illustration of this point. dTCF functions downstream of the Wingless signaling pathway (*47*, *48*) and is associated with Groucho in the absence of a Wingless signal (*49*). However, upon signaling, the β-catenin cell surface protein Armadillo is modified so that the intracytoplasmic domain is released to the nucleus, where it interacts with dTCF (*48*). The resulting dTCF-Armadillo complex is thought to activate the same target genes that were previously repressed by dTCF-Groucho complexes before Wingless signaling. A somewhat unexpected wrinkle to this story is the observation that CBP does not function as a coactivator in this case, but instead facilitates dTCF-mediated repression. CBP acetylates specific lysine residues in dTCF, and this precludes the binding of the Armadillo coactivator (*50*).

There are additional examples of potential on-off regulatory switches mediated by competition between coactivators and corepressors. Among these are members of the nuclear receptor superfamily, including RAR and TR. In the absence of ligand, both receptors interact with Rpd3 corepressor complexes. Upon binding to ligand, the nuclear receptors are converted into transcriptional activators by interacting with coactivator complexes that contain CBP and related histone acetyltransferases (*12*) or coactivator complexes related to ARC (*19*, *51*). Moreover, the E2F proteins function as activators of S-phase cell-cycle genes, possibly by interacting with the CBP coactivator (*52*). However, at different phases of the cell cycle, the Rb corepressor is dephosphorylated, which allows it to interact with E2Fs, converting them to repressors of S-phase genes (*53*). Rb is thought to mediate repression through two distinct mechanisms: It inhibits sequence-specific activators bound to target promoters (*54*), and it also recruits the Rpd3 histone deacetylase to alter chromatin structure (*55*).

Coregulators and Threshold Gradients in Development

Dorsal is probably the most thoroughly characterized sequence-specific transcription factor in development. It regulates gene expression in a concentration-dependent manner, and a variety of target genes have been analyzed in an effort to determine how the Dorsal gradient establishes different thresholds of gene activity and tissue differentiation (*25*) (Fig. 2). We propose that Dorsal gradient thresholds can be described on the basis of recruiting coactivators and corepressors to target promoters. As discussed previously, the specification of the mesoderm might depend on the recruitment of CBP coactivator complexes to the promoter regions of *twist* and *snail* (Fig. 2). The specification of the neurogenic ectoderm may involve an interplay between CBP, or other coactivators, and the dCtBP corepressor. *rhomboid* is regulated by a distal enhancer that contains tightly clustered binding sites for both Dorsal and bHLH activators. Cooperative interactions between these proteins ensure efficient occupancy of Dorsal binding sites so that low levels of the Dorsal gradient can activate *rhomboid* (*56*). The distal *rhomboid* enhancer also contains four binding sites for the Snail repressor, which is restricted to the ventral mesoderm. The recruitment of both coactivators and dCtBP keeps *rhomboid* off in the ventral mesoderm (Fig. 2). In addition to activating *twist*, *snail*, and *rhomboid* in the mesoderm and neurogenic ectoderm, the Dorsal gradient also works as a repressor to establish the dorsal ectoderm. As discussed previously, the Dorsal gradient keeps *zerknüllt* off in the mesoderm and neurogenic ectoderm by recruiting Groucho to the distal silencer element (Fig. 2). Thus, the specification of the neurogenic ectoderm and dorsal ectoderm depends on two different corepressors, dCtBP and Groucho, respectively.

Summary

There is emerging evidence that sequence-specific activators and repressors interact with coregulators, which in turn either stimulate or inhibit the binding or function (or both) of the Pol II transcription complex.

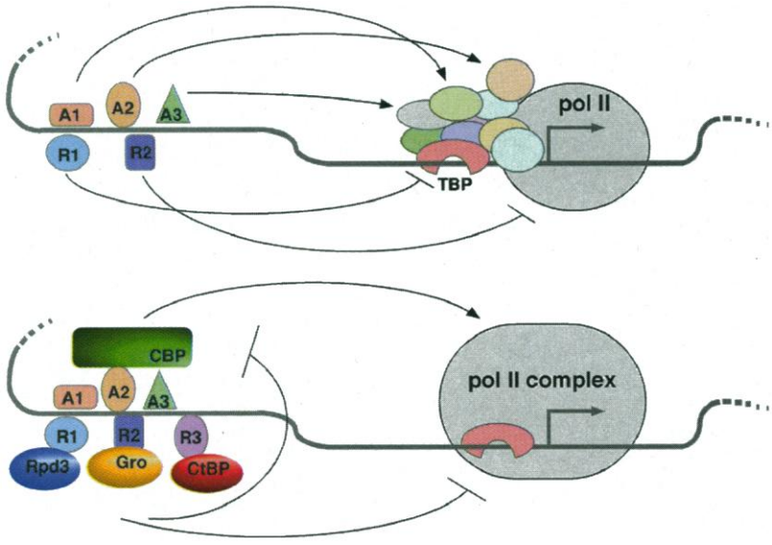

Fig. 3. Integration of combinatorial information. (**Top**) It is possible that the recruitment or assembly of Pol II involves a series of independent interactions of various upstream activators (A1, A2, A3) and repressors (R1, R2, R3) with different components of the Pol II complex. If the activators prevail, then the Pol II complex is brought to the core promoter. According to this model, the core Pol II complex serves as the substrate for integrating the different activators and repressors. (**Bottom**) An alternative model is that activators and repressors locally interact within the limits of a distal enhancer. They subsequently recruit various coactivators and corepressors, which in turn send a simple On or Off signal to the Pol II complex. According to this model, signal integration occurs at the level of cis-regulatory DNA. The two models are not mutually exclusive, and gene regulation could be achieved through a combination of both mechanisms.

These findings, together with the realization that cis-regulatory DNA can be organized in a series of separate modules or enhancers, suggest that the Pol II complex itself may not be the primary substrate for integrating diverse signals in the combinatorial control of gene expression. Although activators and repressors bound along the length of a promoter region might separately interact with different subunits of the Pol II complex (Fig. 3), a nonexclusive alternative view is that cis-regulatory DNA serves as the key substrate for the combinatorial integration of gene regulation. Activators and repressors would locally interact and recruit coactivators or corepressors (or both) to the enhancer. The coregulators subsequently relay a simple On or Off signal to the core promoter by either recruiting or not recruiting the Pol II complex (Fig. 3). In principle, this model can account for the evolution of modular promoter regions and complex patterns of gene expression during development.

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