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PERSPECTIVES: TRANSCRIPTION

Switching Partners in a Regulatory Tango

Kenichi Nishioka and Danny Reinberg

An intricate network of protein-modifying enzymes operates in the transmission of signals from the cell surface to the nucleus. Proteins are most commonly modified by the addition or removal of phosphate groups (phosphorylation/dephosphorylation) or acetyl groups (acetylation/deacetylation) (*1*). Recently, addition of methyl groups (methylation) to proteins has been discovered, although this process may not be reversible (*2, 3*). One of the targets of these three types of modification are the histone proteins that form the beads (nucleosomes) around which the DNA is wrapped. On page 2507 of this issue, Xu *et al.* (*4*) shed new light on how protein methylation regulates gene expression driven by the nuclear hormone receptor (NR) class of transcription factors.

Binding of a gene-specific transcription factor to a target DNA sequence (promoter) initiates recruitment of a plethora of coactivators that are necessary for the gene to be transcribed. Some coactivators interact with only one class of gene-specific transcription factor. For example, members of the p160 family of coactivators collaborate with NRs to initiate transcription. Other coactivators have looser specificities and interact with multiple types of transcription factors as a way of integrating several signal transduction pathways. Examples of such coactivators include the CREB (cyclic adenosine monophosphate response element-binding protein) binding protein (CBP) and its paralog p300, which possess histone acetyltransferase activity. CBP/p300 is recruited to many distinct target genes as a coactivator and initiates gene activation by acetylating histone proteins and associating with the enzyme responsible for transcription, RNA polymerase II (*5*).

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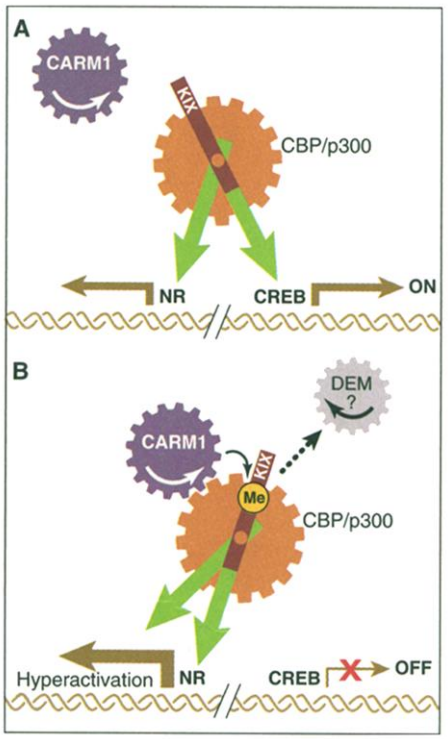
The fact that CBP/p300 has such a general effect raises the question of whether the cell contains enough CBP/p300 to translate signal transduction events into gene transcription at so many promoter sites. Several lines of evidence suggest that both CBP and p300 are present in small amounts and thus are limiting in most cells. For example, patients with Rubinstein-Taybi syndrome, who lack one CBP allele, suffer severe developmental defects (*6*). In another example, HIV gene expression is induced initially by binding of tumor necrosis factor–α (TNF-α) to its receptor on the cell surface. TNF-α stimulates the p65 subunit of the transcription factor NF-κB, which then translocates to the nucleus to activate transcription of the HIV genome. When the Stat2 protein is activated in concert with NF-κB, Stat2 competes with TNF-α–stimulated p65 for binding to CBP/p300, which results in the inhibition of HIV gene expression (*7*). Finally, an analysis of protein partner switching during nerve cell differentiation disclosed that neurogenin interacts with CBP and promotes neuronal differentiation. When the amount of neurogenin in the nerve cell drops, CBP is then free to interact with the Stat3 protein, resulting in the induction of glial cell differentiation (*8*). This suggests that CBP is at the center of a differentiation "switch" that dictates whether progenitor nerve cells will become neurons or glia. Taken together, the results of these three studies suggest that the cellular pool of CBP/p300 is so small that any competition for these proteins has a demonstrable effect on cellular phenotype. But how CBP/p300 switches its partners so smoothly is not clear.



The CARM1 molecular switch. (**A**) Usually CBP/p300 is not methylated and this coactivator complex is able to initiate transcription of both NR- and CREB-dependent genes (green arrows). (**B**) CARM1 methylates (dark blue circle) the KIX domain (red) of CBP/p300, such that CBP/p300 is no longer able to activate CREB-dependent genes. In this case, CBP/p300 is now available to direct NR-dependent gene activation exclusively. A hypothetical demethylase (DEM) might be able to reverse the consequences of CARM1 methylation.

Previous studies showed that the enzyme CARM1 (coactivator-associated arginine methyltransferase 1) binds to the carboxyl-terminal region of members of the p160 family of coactivators. Upon binding, the histone methyltransferase activity of CARM1 enhances NR-dependent gene transcription (*9*). Subsequent work established that CARM1 and CBP/p300 synergistically enhance NR-dependent gene expression (*10*). Now, Xu *et al.* illustrate the molecular basis of the observed synergy between these two transcriptional coactivators, and describe how CARM1 confers gene specificity upon CBP/p300 (*4*). First, the authors show that CARM1 binds directly to CBP/p300 (see the figure). This work suggests that CBP/p300 and CARM1 exist as a coactivator complex in which the histone acetyltransferase activity of CBP/p300 potentiates the his-

tone H3 methyltransferase activity of CARM1, resulting in enhanced NR-dependent gene activation. Furthermore, and most important, Xu *et al.* also show that CARM1 methylates the KIX domain of CBP/p300 (see the figure). Methylation of the KIX domain interferes with the ability of CBP/p300 to interact with CREB's KID motif (*11*), causing the loss of CREB-dependent gene activation (see the figure). Upon methylation, then, the limiting pool of CBP/p300 becomes available for interaction with other transcription factors that regulate, for example, NR-dependent gene transcription.

This transcriptional "switch" from CREB-regulated to NR-regulated gene expression is especially intriguing because the CREB family of transcription factors is crucial for many cellular events, such as glucose homeostasis, growth factor–dependent cell survival, and generation of an immune response. Moreover, the CREB family has been implicated in learning and memory (*11*). Although phosphorylation of CREB is sufficient for it to induce expression of its target genes, additional cofactors, such as CBP, are required for further gene activation in response to mitogens or stress. It remains unclear how phosphorylated CREB discriminates between various cellular signals, although there are several hypothetical ways that CREB's interaction with CBP could be blocked (*11*).

Methylation of CBP/p300 by CARM1 appears to destabilize the structure of the KIX domain, implying that interactions between CBP/p300 and other proteins may also be disrupted. Indeed, Xu *et al.* show that interaction between the transcription factor c-myb and CBP/p300 is lost upon methylation of the KIX domain. Thus, CARM1-mediated methylation of the KIX domain could be one way to modulate the specificity of CBP/p300 binding to gene-specific transcription factors. However, it is necessary to consider the implications of these findings in the context of natural promoters—such as the promoter for the retinoic acid receptor–β (RAR-β) gene—on which CREB, RAR, and another nuclear hormone receptor, RXR, form a complex that regulates transcription of this gene.

The existence of the CREB-NR molecular switch raises some additional questions. Does methylation of the KIX domain of CBP/p300 impose an irreversible signal that dictates which genes are to be transcribed? Does the activation of such genes require stepwise signaling at the promoter? If so, then how is the promoter to be silenced when new environmental conditions present themselves? Is arginine methylation of CBP and surrounding nucleosomes a reversible reaction, or does methylation of the KIX domain mark CBP/p300 for destruction? If so, is there any link between arginine methylation and ubiquitination? Several points also remain to be addressed regarding the regulation of CARM1 itself: What signal activates CARM1, and how does CARM1 activate NR-dependent but not CREB-dependent transcription? How are these signals integrated at composite promoters where transcription depends on both CREB and NR? Clearly, with so many potential points of regulation, gene expression will continue to unveil new steps in its elaborate dance.

The observation that CARM1 is an essential part of a molecular switch that determines whether CBP/p300 is to be used for NR-dependent or CREB-dependent gene activation allows us to speculate on ways this molecular switch can be exploited for medical purposes. For example, drugs that can demethylate proteins and so specifically antagonize CARM1 activity could potentiate CREB-dependent gene activation. Such antagonists might enhance long-term potentiation in neurons and hence improve learning and memory, or may boost the immune response in immunosuppressed individuals. In contrast, induction of CARM1 may increase sensitivity to nuclear hormones or raise an organism's threshold to stress signals. In any case, this new molecular switch will provide fascinating insights into the sophisticated mechanisms of gene regulation.

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NOTA BENE: DEVELOPMENT

A SAC of Crumbs and Stardust

Epithelial cells like to know which way is up. They exist in neat rows, one on top of another, each cell bound tightly to its neighbor through protein complexes (junctions) that unite the cells and allow them to communicate. It is the segregation of these protein complexes to specific regions of the plasma membrane that enables epithelial cells to distinguish top from bottom. This cellular polarity ensures that epithelial tissues retain their highly organized architecture.

But what events in the embryo trigger epithelial cells to acquire this polarity? Work in fly embryos established that a transmembrane protein called Crumbs becomes localized at the apex of epithelial cells early in development. Two groups reporting in *Nature* (*1*, *2*) now reveal that Crumbs is not the lone director of apical polarity in fly epithelia. Clinging to the cytoplasmic tail of Crumbs is an accomplice called Stardust. Each protein depends for its localization and stability on the other, and loss of either protein dooms epithelial tissues to a disorganized existence.

The lateral surfaces of a fly epithelial cell contain three junctions: the subapical complex (SAC), closest to the top of the cell; the zonula adherens, in the middle; and beneath it, the septate junction. Both groups show that during gastrulation, Stardust, like Crumbs, becomes localized to the apicolateral region of the epithelial cell plasma membrane where the SAC forms. If Stardust is absent, the SAC does not form and is unable to direct assembly of the zonula adherens. The result is loss of epithelial cell polarity and the progressive disorganization of epithelia as embryogenesis progresses.

Discovering Stardust in fly sensory neurons, as well as in epithelial tissues, suggested to the investigators that Stardust could be involved in the polarity of embryonic neuroblasts, cells derived from epithelia that eventually form the fly's central nervous system. Intriguingly, it turned out that neither Stardust nor Crumbs seemed to be involved in neuroblast polarity, a task left to the Bazooka–Dm-Par-6–aPKC triumvirate of proteins. Stardust, however, accumulates in the dendritic tips of sensory neurons such as those of fly stretch mechanoreceptors (*1*). The researchers propose that Stardust may lead a duplicitous existence, directing epithelial cell polarity in the fly during early embryogenesis, but contributing to mechanosensory transduction later in development. –ORLA SMITH

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