derepressed IIIb to interfere with IIIc inclusion in hMSCs. Luco et al. (2010) next extended the analysis genome-wide, showing that a significant portion of splicing events regulated by PTB were also regulated by MRG15. The authors found that the splicing events regulated by both PTB and MRG15 were similarly affected in response to RNAi against PTB or MRG15. A complete overlap between PTB and MRG15 targets is not expected because many PTB-dependent events may not need additional modulation by MRG15, whereas other MRG15-dependent events might be coupled with different splicing regulators. These questions can now be further pursued by comparing genome-wide MRG15 binding with the recent genome-wide map of PTB binding on RNA (Xue et al., 2009). Interestingly, H3K4me3 distribution across genes exhibits an opposite profile to H3K36me3 between PNT2 and hMSCs, and overexpression of a methyltransferase for H3K4me3 reduced IIIc inclusion by a mechanism that remains elusive. Furthermore, H3K9me1, a histone mark generally linked to gene repression, is selectively enriched on IIIb in PNT2 cells relative to hMSCs, raising the possibility that RNA polymerase II pauses at IIIb to favor its selection in epithelial cells. These observations leave open a long list of questions to be pursued in future studies.

Collectively, the findings of Luco et al. (2010) demonstrate a clear link between chromatin features and regulated splicing. We may be looking at just the tip of the chromatin modification iceberg, considering the potential combinatory influence of nucleosome positioning on the kinetic coupling between transcription and splicing, appearance of specific *cis*-acting elements from nascent RNA, and recruitment of splicing regulators that may act in a position- and context-dependent manner. Clearly, complete elucidation of the splicing code must now consider the contribution of the histone code. Indeed, it has been reported that there is increased accuracy in the prediction of splice site usage when information about nucleosome enrichment is added to exon prediction programs (Spies et al., 2009). However, the increase was relatively small, suggesting a long journey ahead of us in predicting the splicing code.

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# BCL6 and BCoR Gang Up on Notch to Regulate Left-Right Patterning


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**In this issue of *Developmental Cell*, Sakano et al. describe a novel mechanism of how a key lymphocyte transcription factor crosstalks to Notch signaling during embryonic development and thereby selectively inhibits Notch-activated target genes to allow proper left-right patterning.**

The Notch signaling pathway is recurrently used during development in many cell types and tissues to regulate processes such as differentiation, proliferation, and survival. The signaling cascade appears to be very simple: binding of extracellular ligands to Notch receptors on neighboring cells induces the proteolytic cleavage and release of the Notch intracellular domain (NICD). NICD translocates to the nucleus, heterodimerizes with the transcription factor CSL (CBF-1 for humans, Suppressor of hairless for *Drosophila*, and LAG-1 for *Caenorhabditis elegans*, also known as RBP-J in the mouse), and recruits coactivators, including Mastermind-like proteins, to induce transcription of target genes (Bray, 2006). A multitude of Notch target genes have been identified, some of which are cell type specific, while others are activated in many cell types and developmental processes. How Notch signaling activates only selected target genes

while others are not activated despite the presence of CSL binding sites in their promoters or enhancers is mostly unknown. In this issue of *Developmental Cell*, Sakano et al. (2010) identify the transcriptional repressor B cell leukemia/lymphoma 6 protein (BCL6) as a protein that interacts with components of the Notch-specific transcription machinery to selectively repress certain Notch target genes to allow proper establishment of left-right (LR) asymmetry during *Xenopus* development.

On the exterior, vertebrates are essentially bilaterally symmetrical; however, their interiors exhibit multiple asymmetries, exemplified by the LR-asymmetric positions of many organs and the coiling of the intestine (Raya and Izpisúa Belmonte, 2006). The critical elements in breaking symmetry take place at the node, an important organizer structure; LR asymmetry is subsequently transduced to the lateral plate mesoderm (LPM), which serves as a global conveyor of LR-asymmetric information along the body axis. During establishment of LR asymmetry, *Nodal* expression around the node is directly activated by Notch signaling from a specific enhancer element present in the *Nodal* gene (Krebs et al., 2003; Raya et al., 2003). This process is a crucial step in initiating LR asymmetry, because diverse mutations affecting the Notch pathway cause LR patterning defects characterized by the absence of Nodal expression in the perinodal region and the LPM (Krebs et al., 2003; Raya et al., 2003). Moreover, Nodal initiates the expression of *Pitx2*, a transcription factor essential for LR patterning in the LPM (Raya and Izpisúa Belmonte, 2006). However, *Pitx2* expression is also induced in Notch mutant embryos lacking Nodal function, indicating that *Pitx2* is regulated by both Nodal-dependent and -independent mechanisms (Krebs et al., 2003; Raya et al., 2003). It is conceivable that Notch signaling is needed at the initial phase of LR patterning to induce Nodal expression, whereas at later stages Notch needs to be inhibited to allow *Pitx2* expression.

To gain further insight into how Notch signaling might regulate transcription during embryogenesis, Sakano et al. (2010) used an immunoprecipitation strategy followed by mass spectrometry analysis to identify novel transcriptional regulators that can interact with the ankyrin repeat domain of NICD. The authors thereby identified BCL6 as a NICD-interacting protein. BCL6 is a transcriptional repressor that was first identified as a proto-oncogene frequently expressed in non-Hodgkin's lymphomas as a consequence of chromosomal translocations (Pasqualucci et al., 2003). Genetic mouse studies revealed important functions for BCL6 during T and B lymphocyte differentiation, including the regulation of germinal B cell differentiation to generate long-lived antibody-secreting plasma cells from antigen-specific B cells (Crotty et al., 2010).

Sakano et al. (2010) performed elegant Morpholino-mediated knockdown and rescue experiments and thereby established a role for BCL6 in LR patterning of *Xenopus* embryos. Moreover, they linked BCL6 to the Nodal-Pitx2 axis and showed that BCL6 inhibits Notch signaling, which is necessary to maintain *Pitx2* expression. BCL6-mediated inhibition of Notch signaling is achieved by direct binding to NICD and by association with corepressors such as BCL6-corepressor (BCoR). This process prevents the recruitment of MAM1 (the ortholog of Mastermind-like proteins) into the Notch-specific transcription complex. Most notably, the authors showed that BCL6 is not a general inhibitor of Notch signaling, but instead selectively inhibits certain Notch target genes such as *enhancer of split related 1* (*ESR1*) while other target genes including *Hairy2* remain activated. The specificity of Notch target gene inhibition is mediated by direct binding of BCL6 to promoter and/or enhancer elements of the corresponding gene (in this case *ESR1*).

The importance of this publication goes beyond establishing a novel role for BCL6 in LR patterning of *Xenopus* embryos. The studies by Sakano et al. establish one of the rare mechanistic explanations for how transcription of selected Notch target genes can be inhibited, while others remain activated, in order to establish a proper developmental process. They convincingly show that BCL6/BCoR acts as a competitor for MAM1 in the protein complex regulating *ESR1* transcription and thus confines the Notch signal to cell-specific target genes. Future studies will be necessary to investigate how the Notch-ESR1 axis regulates *Pitx2* gene expression in more detail. It is however conceivable that such a mechanism is not only restricted to LR patterning but might find its conservation in tissues where Notch and BCL6 expression patterns overlap to define specific developmental, homeostatic, or pathophysiological processes.

It is worth noting that Notch, BCL6, and BCoR have been linked to overlapping sets of human leukemias and congenital disorders. Correlative evidence connecting BCL6 and Notch to human disease was observed in diffuse large B cell lymphoma, where Notch2 gain-of-function mutations and increased BCL6 protein expression are common (Lee et al., 2009). In Hodgkin's lymphoma cell lines, BCoR is detected at a number of BCL6 target genes, implying that BCoR might play a role in mediating lymphomagenesis (Pasqualucci et al., 2003). In addition, mutations in the *BCoR* gene can cause Oculofaciocardiodental syndrome, which includes features of LR patterning defects such as intestinal malrotation, asplenia, and dextrocardia (Hilton et al., 2007; Ng et al., 2004). These findings suggest that some functions of BCL6/BCoR transcriptional regulation are conserved and may relate to Notch-dependent processes in particular. The novel mechanism described by Sakano and colleagues to direct Notch-specific target gene regulation via BCL6 thus opens new and exciting avenues of research to assess whether these regulatory mechanisms will have a broader impact on other developing, self-renewing, or cancerous tissues.

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# Switch NFix Developmental Myogenesis

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**During development, skeletal muscles adapt to stage-specific functional and metabolic challenges by switching the expression of specific subset of genes. The mechanism that governs these changes is still enigmatic. In a recent issue of *Cell*, Messina and coworkers shed light on this issue through the identification of a transcription factor—NFix—that coordinates the switch in gene expression at the transition from embryonic to fetal myoblasts.**

Every transition in life requires that the preexisting status be erased prior to stepping into a new stage. For instance, it is notoriously difficult to move into a new relationship if the previous one has not been resolved. An analogous situation applies to embryo development, during the transition from one stage to another. Skeletal myogenesis occurs in successive steps, each of them involving distinct progenitor cell types and specific patterns of gene expression (Bryson-Richardson and Currie, 2008). The first muscle fibers in the embryo appear by day e11 from the fusion of embryonic myoblasts. By day 16 a second wave of myogenesis is driven by fetal myoblasts, which give rise to most of the adult musculature. In postnatal life, muscle growth and regeneration occurs at the expense of a heterogeneous population of adult muscle progenitors—the satellite cells. Embryonic, fetal, and adult muscle progenitors show different patterns of gene expression that comply with stage-specific activities (Gunning and Hardeman, 1991). Switching on or off specific subsets of genes at each transition is therefore a critical challenge faced by developmental myogenesis. Despite the knowledge of the molecular networks that specify the myogenic lineage and activate skeletal myogenesis by the cooperative activity of different transcription factors (Guasconi and Puri, 2009), the identities of the cellular factors that coordinate gene repression and activation at each transition remains elusive. In a recent *Cell* paper, Messina et al. (2010) demonstrate that a single transcription factor—nuclear factor I-x (Nfix)—is necessary and sufficient to mediate the transcriptional switch between embryonic and fetal myogenesis.

Nfix belongs to a class of transcription factors consisting of four closely related genes—Nfia, Nffb, Nfic, and Nfix—that are involved in the control of gene expression in a variety of cell types and tissues (Gronostajski, 2000). Nfi-binding sites have been implicated both in gene activation and repression, but the mechanism by which they modulate transcription is still obscure. Nfi proteins bind to DNA either as homodimers or heterodimers with other family members through an N-terminal region; the C-terminal region is highly variable, as the result of extensive alternative splicing, and contains domains responsible for transcriptional activation or repression (Gronostajski, 2000). Mouse models in which the expression of the different family members has been ablated have revealed the role of Nfia and Nfib in brain development (with Nfib being also essential for lung maturation) and of Nfic in correct tooth formation. Nfix-deficient mice die soon after birth with defects in brain, intestine, and skeleton (Pekarik and Belmonte, 2008). Thus, the discovery by Messina et al. (2010) that Nfix has a crucial role in skeletal myogenesis is unanticipated. A genome-wide screen in fetal versus embryonic myoblasts, previously performed by the same group, showed an abundant and preferential expression of Nfix in fetal myoblasts (Biressi et al., 2007). Messina et al. (2010) now use a combination of assays in established and primary (embryonic or fetal) mouse muscle cells to demonstrate the role of Nfix in the activation of gene expression typical of fetal myoblasts. In vivo experiments show that conditional ablation of Nfix in MyoD-expressing cells prevents the initiation of fetal-specific transcription. Consistently, premature expression of Nfix in embryonic myoblasts leads to an anticipated activation of fetal genes and suppression of embryonic genes. This evidence led the authors to conclude that Nfix coordinates gene expression