of these particles increased 3- to 4-fold during flagellar shortening (Pan and Snell, 2005). These investigators demonstrate that the few cilia that form in cells partially depleted of IFT88 protein (known to be essential for ciliary assembly) are hindered during serum-stimulated shortening, and that inhibition of AurA alters the localization of IFT88 accompanying serum stimulation. Thus, signaling involving IFT might also be coupled to ciliary shortening in mammalian cells.

Does the failure to enter mitosis in cells with experimentally reduced activity of AurA, HEF1, or HDAC6 reflect a regulatory system that monitors whether the centriole has been liberated from its role as a basal body? Few studies, if any, have addressed a possible role of ciliary shortening/centriole liberation in the cell cycle in multicellular organisms. Most of these studies have been done either with ciliogenic cells under experimental conditions in which they do not form a primary cilium or with cells (e.g., HeLa cells) that apparently do not form a primary cilium. Given that tubacin and other tubulin-directed small molecules are being used to treat cancer patients, it will be important to determine whether these agents inhibit the growth of tumor cells due to disruption of ciliary shortening and the cilia-centriole-centrosome cycle.

This recent work also brings into focus a noteworthy but largely unstudied process in the cell cycle—the binding and subsequent release of the basal portion of the ciliary membrane by the distal edge of the centriole (Figure 1). Future studies of this process and on the pathway for cilium shortening have the potential to yield important new insights into cellular and molecular mechanisms that regulate cell proliferation.

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HOTAIR Lifts Noncoding RNAs to New Levels

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It is not clear to what extent noncoding RNAs regulate the homeobox (*HOX*) genes that encode key regulators of development in the embryo. In this issue, Rinn et al. (2007) characterize noncoding RNAs that regulate *HOX* genes and discover one, HOTAIR, that unexpectedly regulates a *HOX* gene cluster on a different chromosome than the *HOX* cluster that encodes it.

"A thing long expected takes the form of the unexpected when at last it comes," Mark Twain once wrote. A striking example of this notion is provided in this issue by Rinn et al. (2007). It has long been thought that HOX gene clusters, key players in embryonic development, are regulated by *cis*-acting noncoding RNAs (ncRNAs). In their new work, Rinn et al. use a high-resolution tiled microarray to systematically identify and characterize hundreds of ncRNAs transcribed from the *HOX* gene cluster of 11 human fibroblast cell lines isolated from distinct positions along the anterior-posterior and proximal-distal body axes. They home in on an intriguingly positioned ncRNA they call HOTAIR and make the surprising discovery that

this ncRNA acts in *trans* to regulate a *HOX* gene cluster on a completely different chromosome, not the *HOX* gene cluster that encodes it.

The *HOX* gene cluster serves as a classic model of gene regulation during embryonic development. The 39 human *HOX* genes are contained within four clusters, called *HOXA–D*, on four independent chromosomes. The clusters are organized similarly which strongly suggests that they have arisen through gene duplication. During development, the expression of a gene within the linear organization of each *HOX* cluster correlates with body position, an unusual situation that suggests conservation of the regulatory mechanisms of the four clusters. Proteins responsible for establishing epigenetic modifications are known to regulate *HOX* gene expression, and it has long been proposed that ncRNAs guide the epigenetic regulation of these genes (Rank et al., 2002, Petruk et al., 2006 and references therein). The HOX proteins themselves are master regulators of other transcription factors, and their overall expression profiles are tightly regulated spatially and temporally. Functionally, the *HOX* genes are critical for correct patterning of the axes during development. The significance of correct *HOX* gene expression was first observed in *Drosophila*. Since then, *HOX* gene clusters have been observed in all metazoans and have similar roles in axial development (reviewed in Lemons and McGinnis, 2006).

Differential *HOX* gene expression is established early on in the developing embryo. Once established, the *HOX* gene expression profile needs to be maintained even as the embryo continues through development. The Polycomb group (PcG) and Trithorax (TrxG) group protein complexes maintain this form of cellular memory as regions of silenced or active transcription, respectively (Klymenko and Muller, 2004). The PcG complexes are associated with trimethylated K27 on histone H3, a modification frequently required for this epigenetic silencing. The TrxG complex is associated with trimethylated K4 on histone H3 which is associated with genes that are actively transcribed.

In addition to the *HOX* gene expression profiles of the human adult fibroblast cell lines originating from different axial positions, Rinn et al. (2007) describe the discovery of many ncRNAs expressed from these fibroblasts. Previously detailed transcriptional analyses of human *HOX* clusters revealed the presence of ncRNAs, many of which have been implicated in transcriptional regulation of neighboring *HOX* genes (Mainguy et al., 2007, Sessa et al., 2007). By combining the powers of a high-throughput high-resolution tiled microarray and bioinformatics algorithms, Rinn et al. (2007) identified nearly 30 kb of new ncRNAs. Similar to the ncRNAs found by other groups, the majority of these transcripts are intergenic and are transcribed in the direction opposite to the *HOX* genes. By having such a deep database, however, these investigators were able to demonstrate that the ncRNAs are also differentially expressed in different cell types according to their original position along the body axis, and, moreover, sequence motifs could be assigned to unique cell types.

Given the flurry of studies on *HOX* to map potential epigenetic regulatory marks on histones (Bernstein et al., 2006, Lee et al., 2006, Boyer et al., 2006), the authors asked whether there were any correlations between epigenetic marks and the location of ncRNAs. Chromatin immunoprecipitation (ChIP)-microarray experiments revealed, not surprisingly, that RNA polymerase II and trimethylated H3K4 (a mark of active transcription) occupied the same regions as the ncRNAs. These were mutually exclusive on the *HOX* clusters with the localization of Suz12 and trimethylated H3K27, a mark of repressed chromatin. This was elegantly shown at the *HOXA* cluster, where a boundary between *HOXA7* and *HOXA9* delineates a clear breakpoint between oppositely acting covalent marks in two distinctly regulated fibroblasts from two different axial positions.

The surprise came, however, when Rinn et al. (2007) characterized one well-placed ncRNA in this region. This 2158 nucleotide RNA, termed HOTAIR, was localized to a regulatory boundary in the *HOXC* cluster and was expressed in both distal and posterior fibroblasts. If HOTAIR was similar to other previously characterized ncRNAs, it might have had a proximal regulatory role (Sessa et al., 2007). However, knockdown of HOTAIR showed no changes in the *HOXC* cluster, but instead showed a loss of transcriptional repression from a 40 kb region of the *HOXD* cluster. In addition, the repressive mark, trimethylated histone H3K27, and the regulatory complex that produces this mark, the PcG complex PRC2, were no longer present at that particular region of the *HOXD* cluster when HOTAIR was depleted. Pull-down experiments of PRC2 components demonstrated a direct and specific interaction with HOTAIR. The observation that this ncRNA binds to an epigenetic regulatory complex and changes methylation patterns on a different HOX cluster on a different chromosome is unexpected.

There are many questions that remain concerning how HOTAIR silences the 40 kb region of the *HOXD* locus. Is this silencing effect direct or indirect? The interaction of HOTAIR with PRC2 is consistent with a direct effect but does not rule out indirect effects. Would ectopic expression of HOTAIR in another fibroblast be sufficient to suppress the *HOXD* locus? The association of HOTAIR with PRC2 also suggests that the mechanism is epigenetic, but this remains an open question. Furthermore, nature tends to use mechanisms more than once: are there other *trans*-acting ncRNAs from the *HOX* cluster or elsewhere that have not yet been found? The characterization of HOTAIR raises intriguing questions about how ncRNAs work and adds to the functional complexity of this class of regulatory RNAs.

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