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Insights Into Cornelia de Lange Syndrome From the Nipbl-Mutant Mouse

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genes in microarray analyses performed on MEFs (mouse embryonic fibroblasts; >30 transcripts) and embryonic brain (>700 transcripts). Importantly, although changes in transcript levels are significant statistically and reproducible over large numbers of individual samples, most changes are relatively small in magnitude, usually less than twofold. Included in this category is the *Nipbl* transcript itself, which is ubiquitously expressed and which was found to be downregulated by ~30% in both adult and embryonic tissues derived from *Nipbl*$^{564/+}$ mice. In contrast, we found no evidence for elevated PSCS (precocious sister chromatid separation) in any cells derived from *Nipbl*$^{564/+}$ mice (fibroblasts, embryonic stem cells, and splenocytes), suggesting that cohesion defects do not occur, or if they do, are very subtle. Altogether, our data support the idea that the phenotypes observed in the *Nipbl* heterozygous mouse, and by extension in CdLS, are the consequence of subtle dysregulation of the expression of numerous genes, which results from a decrease in *Nipbl* function.

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Modeling Early Developmental Defects in Cornelia de Lange Syndrome Using the Zebrafish

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Nipbl (the ortholog of *Drosophila Nipped-B*) has been identified as a gene in which heterozygous mutations are associated with at least 50% of cases of Cornelia de Lange Syndrome (CdLS). *Nipbl* and its relatives encode cohesin regulatory proteins, which are thought to be involved in sister chromatid cohesion and DNA repair. Studies in *Drosophila* have revealed that *Nipped-B* also plays roles in developmental gene expression, possibly by influencing the strength of long-range promoter–enhancer interactions. Our recent studies in *Nipbl*+/− mutant mice strongly support the notion that widespread transcriptional dysregulation underlies the developmental abnormalities in CdLS. However, the precise targets of *Nipbl* and the mechanism(s) by which it regulates gene expression remain unclear. To address these questions, we have chosen the zebrafish (*Danio rerio*) as a model system in which gene expression levels are easily manipulated by injecting morpholino antisense oligonucleotides (MO) and/or in vitro synthesized mRNA. Zebrafish have two *Nipbl* orthologs (designated *zNipbl-1* and *zNipbl-2*), and both genes are expressed ubiquitously during embryogenesis. Predicted amino acid (a.a.) sequences reveal that the N-terminal 200 a.a. and the C-terminal 1,800 a.a—the latter of which contains multiple HEAT domains—are highly conserved (66–80% identical) among zebrafish and mammalian proteins. MO knockdown in fish embryos, using MOs directed against both *zNipbl1* and *zNipbl2*, causes a variety of developmental defects, including abnormalities in heart and gut development, which overlap with abnormalities seen in CdLS. In *Nipbl*-MO-injected embryos (z*Nipbl*-morphants), migration of cardiac progenitors is delayed, leading to cardia bifida (split heart) in about 15% of morphants. In addition, morphants exhibit reduced expression of an endodermal marker, *foxA3*, which correlates with later mis-localization of visceral organs (liver and pancreas) as well as abnormal gut looping. Such abnormalities of visceral organs are also common findings in CdLS. Consistent with the observations of aberrant morphology of heart and visceral organs in *zNipbl*-morphants, microarray and in situ hybridization analyses of gene expression reveals reduced expression of other endodermal genes (*gata5*, *sox32*, *sox17*, and *foxA2*). Although these endodermal genes are all known to be regulated by Nodal signaling, expression of *Nodal* genes, as well as the Nodal mesodermal target genes *no tail* and *goosecoid*, are not affected by *zNipbl*-MO, suggesting that at least some endodermal genes are directly regulated by *Nipbl*. These results suggest a mechanism by which *Nipbl* regulates endodermal development and a hypothesis for the etiology of heart and gut abnormalities observed in individuals with CdLS. We are further testing this hypothesis by examining endodermal gene expression in *Nipbl*-heterozygous mutant mice. Moreover, we are also examining functional relationships between *Nipbl* and cohesin on gene expression by simultaneously manipulating cohesin expression in zebrafish embryos.

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Cohesin-Dependent Regulation of Gene Expression in Zebrafish

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Cohesion or "pairing" of sister chromatids is essential to ensure each daughter cell receives the correct number of chromosomes during cell division. Sister chromatid cohesion is mediated by cohesin, a multimeric protein complex comprising at least four subunits: Rad21/Scc1, SA/Scc3, Smc1, and Smc3. We identified the cohesin subunit *rad21* in a zebrafish forward genetic screen for positive regulators of *runx1*.

Runx transcription factors are critical for determining cell fate in many cell types, and maintaining balanced levels of Runx protein is essential for normal development. Deregulated expression of *runx* genes leads to cancers and developmental disorders. 12-somite zebrafish embryos mutant for *rad21* or morphant for Smc3 lack hematopoietic expression of *runx1*, and fail to express *runx3*. Partial failure of blood development in *rad21* mutants is rescued by microinjection of *runx1* mRNA. Significantly, loss of just one copy of the *rad21* gene caused a reduction in *runx1* transcription and reduced expression of neuronal genes *ascl1a* and *ascl1b*. *rad21* mutant embryos also have neurodevelopmental abnormalities, consistent with the idea that neuronal development is particularly sensitive to loss of cohesin function.