of histone methylation on transcription is not limited to H3K4me3 as a well-known silencing mark, H3K9me3, has been recently linked to gene activation[32]. It is clear that methylation of a particular lysine residue *per se* does not dictate transcriptional outcome. Instead, this is determined by the effector protein or protein complex that binds the methylated residue.

If the above notion is correct, identification and characterization of the effector proteins is crucial for understanding the function of histone modifications. In this sense, the studies described here provide new insights into the function of H3K4 methylation[5–8], but also raise several important questions. (i) How do proteins with modules capable of binding H3K4me3 find their target genes, given that so many different proteins can bind H3K4me3? (ii) How much does the H3K4me3-binding property contribute to target gene recognition? (iii) What other factors are also important for target gene recognition? (iv) Is the moderate difference in binding affinity of PHD fingers for H3K4me2 and H3K4me3 *in vitro* relevant *in vivo*? These questions are pertinent, because NURF, for example, can be recruited to specific target genes through interaction with sequence-specific transcription factors, such as the ecdysone nuclear receptor (EcR)[19]. Interestingly, EcR can also directly recruit an H3K4 methyltransferase coactivator complex[33]. It seems that EcR is upstream in the regulatory pathway whereas NURF and the H3K4 methyltransferase recruitment are downstream. It is likely that recruitment of NURF to a specific gene promoter is mainly determined by sequence-specific transcription factors. Once recruited, it is stabilized by binding to H3K4me3, a mark enriched in many active gene promoters[16,17].

The H3K4me-binding proteins described above are involved in diverse biological processes, including transcriptional regulation, the cell cycle and apoptosis, yet they recognize the same modification. Many of the questions mentioned above could be addressed by a chromatin immunoprecipitation–coupled genomic microarray approach, which would reveal the colocalization of H3K4 methylation sites and specific H3K4me-binding proteins. The relative importance of H3K4me3 in the recruitment of the binding protein could be evaluated by using a similar approach, but analyzing cells that lack H3K4me3, such as cells depleted of WDR5. As both BPTF and ING2 function in complexes with other associated proteins *in vivo*, understanding the potential effect of the associated proteins on H3K4me3 recognition is also important. Answers to many of the above questions are probably forthcoming.

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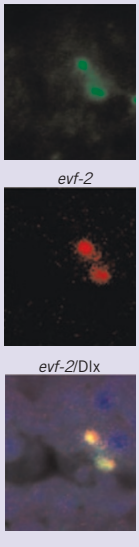
Noncoding RNAs and homeodomains get together

Non-coding RNAs (ncRNAs) exist in many flavors and facilitate a variety of molecular processes, from X chromosome inactivation to splicing to translational regulation. In a recent paper by Kohtz and colleagues (*Genes Dev.* **20**, 1470-1484, 2006), a ncRNA is directly implicated in gene regulation by homeodomain transcription factors during vertebrate brain patterning.

The authors isolated a non-coding transcript, *evf-2*, corresponding to an ultraconserved region (> 90% conserved from fish to humans) that lies between the homeodomain transcription factors Dlx-5 and Dlx-6. *evf-2* is a novel splice form of *evf-1*, a non-coding RNA previously isolated from specific regions of the brain, and is a polyadenylated, single stranded RNA. Although the authors do not exclude the possibility of an *evf-2*–encoded peptide, the RNA contains few open reading frames, suggesting that it is indeed a ncRNA.

evf-2 expression is responsive to sonic hedgehog (shh), a signaling molecule that plays a major role in developmental patterning, as viral overexpression of shh in mouse forebrains results in increased *evf-2* expression. In addition, expression of a reporter carrying the *dlx-5/6* region is increased dose-dependently by the *evf-2* RNA, in a fashion dependent upon the Dlx-2 homeodomain protein. This data is consistent with *evf-2* acting together with Dlx-2 to regulate transcription.



Reporter-based experiments suggest that the effect of *evf-2* on Dlx-2 transcription is specific to the *dlx-5/6* enhancer and cell line specific, perhaps suggesting tissue-specificity. Moreover, the effect of *evf-2* on gene expression is strongest in combination with Dlx-2; *evf-2* has less effect on regulation by other Dlx proteins and little effect on other homeodomain proteins.

Further experiments using the *dlx-5/6* reporter assay indicate that *evf-2* does not repress known Dlx-2 inhibitors, so the possibility of a direct interaction between the ncRNA and the homeodomain protein was tested. Dlx-2 forms a complex with *evf-2* in cells, and *evf-2* can be detected in immunoprecipitates of Dlx-family proteins from embryonic nuclear extracts, suggesting an *in vivo* interaction. In addition fluorescent *in situ* hybridization detects two *evf-2* foci that colocalize with Dlx-2 in cell nuclei within a specific region of the developing mouse forebrain (see picture). While further experiments are required, these data tantalizingly suggest a direct interaction between the two factors.

It is known that fly *Rox* RNAs upregulate male X chromosome transcription, while the *SRA* ncRNA increases steroid receptor gene transcription. Further work will reveal whether there is a common mechanistic basis for ncRNA activity in these different systems and whether other genomic regions contain ncRNAs that regulate transcription of nearby genes.

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