PERSPECTIVE

Balancing import and export in development

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From HOX cofactors to HOX “cofactor complexes”

During the development of multicellular organisms, the *Hox* gene family is involved in axial specification of many different structures, ranging from the neural tube to the intestinal tract. Despite the molecular cloning of the first *Hox* genes in 1984 and finding that they all encode homeodomain-containing transcriptional regulators, there is still little known about the mechanisms by which HOX proteins control gene expression and the nature of the downstream target genes that generate the morphological diversity.

Recently, three evolutionarily conserved classes of related homeobox genes that encode HOX cofactors have been identified (for a recent review, see Mann and Affolter 1998). The PBC proteins [in *Drosophila* encoded by *extradenticle* (*exd*), in vertebrates by the three *pre-B cell homeobox* (*pbx*) genes] can bind cooperatively with HOX proteins to a bipartite DNA sequence and modify the target selectivity, the binding affinity, and the regulatory activity of homeotic proteins. More recently, the PBC proteins themselves were shown to form stable heterodimeric complexes with proteins encoded by two related gene families, the *prep* and *meis* family [*homothorax* (*hth*) in *Drosophila* is a *meis* ortholog]. In contrast to the HOX–PBC interactions, which require binding of the proteins to DNA, the interaction of PBC proteins via the amino-terminal PBCA domain with PREP or MEIS proteins is highly efficient in solution in the absence of DNA. The functional association of HOX proteins with what appear to be extremely stable heterodimeric complexes of PBC and PREP/MEIS proteins has temporarily shifted interest from HOX proteins to these ‘cofactor complexes’ and the elucidation of their functional properties.

Several intriguing properties of PBC and PREP/MEIS proteins were discovered in genetic studies of the corresponding *Drosophila* orthologs *exd* and *hth*. Although first characterized as a HOX cofactor, it turned out that EXD also fulfills important functions in tissues devoid of canonical *Hox* gene products. For example, EXD activity is required for antennal determination (Gonzalez-Crespo and Morata 1995; Casares and Mann 1998). To exert

these functions, *exd* requires the function of HTH. It turns out that EXD activity is regulated post-translationally by a nuclear translocation mechanism: In the absence of HTH, the ubiquitously expressed EXD remains in the cytoplasm and is not functional. In the presence of HTH, EXD translocates to the nucleus, a process that requires direct interaction between EXD and HTH (Rieckhof et al. 1997; Kurant et al. 1998; Pai et al. 1998). The finding that artificial nuclear localization of EXD at ectopic positions does not mimic the phenotypes observed in the presence of ectopic HTH (Casares and Mann 1998) strongly suggests that HTH is not only required for the nuclear localization of EXD but that HTH and EXD also function in a heterodimeric complex, presumably in the regulation of a distinct set of target genes. In *Drosophila* development, nuclear accumulation of the complex is spatially regulated in most cases by the transcription of *hth*, resulting in regulated accumulation of HTH and, consequently, in nuclear import of EXD (Rieckhof et al. 1997).

Controlled balance between import and export

But how is the cytoplasm-to-nucleus translocation of EXD controlled by the presence of HTH protein? Two reports in this issue shed light on this question (Abu-Shaar et al. 1999; Berthelsen et al. 1999). Interestingly, the regulation of the intracellular localization of EXD or PBX1 proteins is accomplished by altering the balance of nuclear import versus nuclear export. The EXD and PBX1 proteins contain both a nuclear import signal (NLS) and a nuclear export signal (NES). The NES is required for the cytoplasmic localization of EXD/PBX1 in cells that lack HTH. The balance of import and export is tipped towards nuclear import by heterodimerization of EXD with HTH (or PBX1 with PREP1), mediated by amino-terminal conserved domains found in these two classes of proteins (the PBCA domain and the MH1 domain, respectively; Bürglin 1998). Both studies map a NLS to the homeodomain portion of the PBC protein (EXD or PBX1). However, the part of the proteins to which the putative NES was mapped differs. Abu-Shaar et al. (1999) report that the PBCB domain, a region of ~90 amino acids located between PBCA and the homeodomain and conserved among PBC family members, is responsible for EXD’s cytoplasmic localization in *Dro-*

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sophila imaginal disc cells and in tissue culture. These investigators propose that HTH binding to EXD may induce a conformational change in EXD that alters the nuclear export/import balance by inactivating the NES, thus favoring nuclear import. Berthelsen et al. (1999) find that the sequences in PBX1 that act as NES in *Drosophila* tissue culture overlap the sequences in the PBCA domain that are essential for the interaction of PBX1 with PREP (or HTH). Therefore, these workers propose that heterodimerization with HTH and PREP induces nuclear translocation of EXD and PBX1, respectively, by directly blocking the NES.

The precise sequence requirements and the mechanism responsible for regulated nuclear export of PBC proteins will have to be investigated further: A critical criterion for an NES is its direct interaction with the export machinery. However, these studies show that altering the balance of export and import of a protein (EXD) through heterodimerization with a developmentally regulated factor (HTH) is a novel and powerful mechanism to accumulate precise stoichiometric amounts of two proteins in the nucleus of specific cells. Indeed, during the development of appendages in the developing fly, the precise spatial regulation of the distribution of HTH-EXD complexes in the nucleus is clearly very important (Abu-Shaar and Mann 1998; Casares and Mann 1998).

What does this all mean for the function of homeotic genes?

Does the distribution of nuclear EXD-HTH complexes as it changes during ontogeny contribute to the functional diversity of HOX proteins? In other words, is HOX activity in certain tissues regulated indirectly via the control of the import/export balance of the cofactor complexes? Clearly, some HOX functions do not require EXD (Peifer and Wieschaus 1990; Rauskolb and Wieschaus 1994; Pinsonneault et al. 1997; Percival-Smith and Hayden 1998). In *Drosophila*, the development of the haltere, for example, is under the control of the homeotic gene *Ultrabithorax* (*Ubx*). Haltere development does not require *exd*, consistent with the finding that EXD protein is cytoplasmic and inactive in the center of the haltere imaginal disc (Azpiazu and Morata 1998). It is likely that the expression of specific *UBX*-regulated genes may be directly perturbed by the presence of nuclear EXD-HTH complexes in the haltere field. Thus, we expect that the developmental specificity of HOX function is linked tightly to the spatial regulation and function of the cofactor complexes. It is also possible that the export/import balance of PBC proteins is regulated at the post-translational level in certain cells, for example by cell-cell signaling, thereby influencing the function of HOX proteins (Mann and Abu-Shaar 1996). Clearly, studies on the molecular function of HOX proteins have to be paralleled by a detailed analysis of cofactor requirements.

Very recently, the structures of the *UBX*-EXD and *HOXB1*-PBX1 DNA complexes were published (Passner et al. 1999; Piper et al. 1999). In these complexes, all of

the homeodomains fold into the characteristic three α helix-containing globular domains. While the two homeodomains bind to opposite sides of the DNA, the conserved hexapeptide amino terminal to the HOX homeodomain inserts into a hydrophobic pocket on the PBC homeodomain. This pocket is created partly by a tripeptide loop that is characteristic for the PBC class of homeodomains. The use of these evolutionarily conserved features in HOX and PBC proteins for cooperative DNA binding suggests a strong selective pressure favoring these particular intermolecular interactions. However, the stable heterodimer formation between PBC proteins and PREP/MEIS proteins suggests that in all cases a third homeodomain is linked physically in vivo to the dimeric HOX-PBC complexes. The presence of the characteristic tripeptide loop in the PREP/MEIS homeodomains further suggests the possibility that such a HOX-PBC-PREP/MEIS triple complex interacts on DNA with yet another HOX or HOX-related protein that inserts the hexapeptide into the predicted pocket in the PREP/MEIS component (Shen et al. 1997; Kroon et al. 1998; Swift et al. 1998). Clearly, more and more partners are joining the HOX proteins.

But do all these proteins contribute to HOX function? So far, the formation of triple complexes has only been reported in DNA binding assays in vitro (Berthelsen et al. 1998b). In cell culture experiments, it has been shown that PREP1 is able to regulate positively the transcriptional activity of the *HOXB1*-PBX1 complex on the *Hoxb-1* autoregulatory element (Berthelsen et al. 1998b). The available structures allow the design of specific mutations in interaction surfaces (e.g., the binding pocket) of PBC or PREP/MEIS proteins and the analysis of their effect on *cis*-acting elements known to be direct targets in vivo (Pöppel et al. 1995; Grieder et al. 1997). Similarly, the effect of hexapeptide mutations in vivo on well defined targets will allow the investigation of the function of HOX proteins in the absence of cooperativity. By analogy, mutations in the homeodomains that abolish DNA binding might also shed light on the composition of the complex on in vivo targets. Despite the increasing complexity of these homeoprotein-containing regulatory complexes, characterization of these complexes will eventually help to elucidate the molecular mechanisms underlying the exquisite control of morphological diversity by *Hox* genes during animal development.

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