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Differences in *Krox20*-Dependent Regulation of *Hoxa2* and *Hoxb2* during Hindbrain Development

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Abstract

During hindbrain development, segmental regulation of the paralogous *Hoxa2* and *Hoxb2* genes in rhombomeres (r) 3 and 5 involves *Krox20*-dependent enhancers that have been conserved during the duplication of the vertebrate *Hox* clusters from a common ancestor. Examining these evolutionarily related control regions could provide important insight into the degree to which the basic Krox20-dependent mechanisms, *cis*-regulatory components, and their organization have been conserved. Toward this goal we have performed a detailed functional analysis of a mouse *Hoxa2* enhancer capable of directing reporter expression in r3 and r5. The combined activities of five separate *cis*-regions, in addition to the conserved Krox20 binding sites, are involved in mediating enhancer function. A CTTT (BoxA) motif adjacent to the Krox20 binding sites is important for r3/r5 activity. The BoxA motif is similar to one (Box1) found in the *Hoxb2* enhancer and indicates that the close proximity of these Box motifs to Krox20 sites is a common feature of *Krox20* targets *in vivo.* Two other rhombomeric elements (RE1 and RE3) are essential for r3/r5 activity and share common TCT motifs, indicating that they interact with a similar cofactor(s). TCT motifs are also found in the *Hoxb2* enhancer, suggesting that they may be another common feature of *Krox20*-dependent control regions. The two remaining *Hoxa2 cis*-elements, RE2 and RE4, are not conserved in the *Hoxb2* enhancer and define differences in some of components that can contribute to the Krox20-dependent activities of these enhancers. Furthermore, analysis of regulatory activities of these enhancers in a *Krox20* mutant background has uncovered differences in their degree of dependence upon *Krox20* for segmental expression. Together, this work has revealed a surprising degree of complexity in the number of *cis*-elements and regulatory components that contribute to segmental expression mediated by *Krox20* and sheds light on the diversity and evolution of Krox20 target sites and Hox regulatory elements in vertebrates.

Keywords

Krox20; *Hoxa2*; *Hoxb2*; hindbrain segmentation; rhombomeres; transgenic mice; pattern formation; vertebrate evolution; gene regulation

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