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# Cooperative function of *deltaC* and *her7* in anterior segment formation

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**Abstract**

Segmentation of paraxial mesoderm in vertebrates is regulated by a genetic oscillator that manifests as a series of wavelike or cyclic gene expression domains in the embryo. In zebrafish, this oscillator involves members of the Delta/Notch intercellular signaling pathway, and its down-stream targets, the *Her* family of transcriptional repressors. Loss of function of any one of the genes of this system, such as *her7*, gives rise to segmentation defects in the posterior trunk and tail, concomitant with a disruption of cyclic expression domains, indicating that the oscillator is required for posterior segmentation. Control of segmentation in the anterior trunk, and its relationship to that of the posterior is, however, not yet well understood.

A combined loss of the cyclic *Her* genes *her1* and *her7* disrupts segmentation of both anterior and posterior paraxial mesoderm, indicating that *her* genes function redundantly in anterior segmentation. To test whether this anterior redundancy is specific to the *her* gene family, or alternatively is a more global feature of the segmentation oscillator, we looked at anterior segmentation after morpholino knock down of the cyclic cell-surface Notch ligand *deltaC* (*dlc*), either alone or in combination with *her7*, or other Delta/Notch pathway genes. We find that *dlc* is required for coherence of wavelike expression domains of cyclic genes *her1* and *her7* and maintenance of their expression levels, as well as for cyclic transcription of *dlc* itself, confirming that *dlc* is a component of the segmentation oscillator. Dose dependent, posteriorly-restricted segmentation defects were seen in the *dlc* knock down, and in combination with the *deltaD* or *notch1a* mutants. However, combined reduction of function of *dlc* and *her7* results in defective segmentation of both anterior and posterior paraxial mesoderm, and a failure of cyclic expression domains to initiate, similar to loss of both *her* genes. Thus, anterior segmentation requires the functions of both *her* and *delta* family members in a parallel manner, suggesting that the segmentation oscillator operates in paraxial mesoderm along the entire vertebrate axis.



*Keywords:* *deltaC*; *her7*; *beamter*; *after eight*; *deadly seven*; *deltaD*; Segmentation; Somitogenesis; Oscillator; Notch signaling; Somites

## Introduction

The segmented architecture of the vertebrate embryo and its relationship to segmented structures of the adult have been appreciated for centuries, but the mechanisms whereby this spatial pattern emerges during embryogenesis are only just being deciphered. In all vertebrates, the paraxial mesoderm, which later gives rise to the reiterated skeletal elements and muscles of the adult body, segments through serial formation of blocks of cells called somites from the morphologically unpatterned Pre-Somitic Mesoderm (PSM). A remarkable insight into this process has come from findings in chick, mouse, and zebrafish embryos of dynamic, wavelike gene expression patterns in the PSM (reviewed in Pourquie, 2001; Rida et al., 2004). These expression domains initiate in the posterior PSM with a period equal to the time interval between somite formation, travel anteriorly through the PSM from the tailbud, then arrest at a location in the anterior PSM that predicts the site of future somite boundary formation. The mRNAs and proteins with such expression patterns, products of the so-called cyclic genes, do not themselves propagate through the PSM, nor do cells of the PSM move with similar velocity (Palmeirim et al., 1997). Rather, these cyclic

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expression patterns are thought to represent the coordinated activity of genetic oscillators in each cell in the PSM. Indeed, studies of cyclic *Hes1* gene mRNA and protein oscillations in cultured mouse cells suggest that for several cycles this phenomenon can be maintained in a cell-autonomous manner (Hirata et al., 2002). At present, cyclic expression patterns in the PSM are the only measurement of segmentation oscillator integrity in the embryo. The role of this oscillator in segmentation and its composition and underlying mechanism are the subjects of continuing study.

To date, the cyclic genes (with the exception of *axin2*; Aulehla et al., 2003) are members of the canonical Delta/Notch signal transduction system: either ligands (*deltaC* (Jiang et al., 2000)), modifiers of Notch receptor glycosylation (*Lfng* (Aulehla and Johnson, 1999; McGrew et al., 1998)), or target genes of the activated Notch receptor such as members of the Hairy-Enhancer of Split Related (HER) family of transcriptional repressors (*c-hairy1*, *c-hairy2*, *Hes1*, *Hes7*, *Hey2*, *her1*, *her7*, and *esr9* (Bessho et al., 2001a; Henry et al., 2002; Holley et al., 2000; Jouve et al., 2000; Leimeister et al., 2000; Li et al., 2003; Oates and Ho, 2002; Palmeirim et al., 1997; Sawada et al., 2000)). Furthermore, cyclic gene transcription of *Lfng* in the mouse is dependent on Notch-responsive elements in the *Lfng* promoter (Cole et al., 2002; Morales et al., 2002). This raises the possibility that the action of this signaling system is linked to or even constitutes a part of the oscillatory mechanism.

Loss of cyclic gene function, such as for *Lunatic fringe* (*Lfng*) or *Hes7* in mouse (Bessho et al., 2001b, 2003; Evrard et al., 1998; Zhang and Gridley, 1998), and *deltaC*, *her1*, *her7* in zebrafish (Henry et al., 2002; Holley et al., 2002; Oates and Ho, 2002), results in a segmentation phenotype characterized by irregularly shaped, partial, and bilaterally asymmetric furrow formation. The combined loss of *her1* and *her7* results in the most severe segmental defects in zebrafish, but sclerotome precursors, twitching muscle, and epithelial furrows are nevertheless generated from the paraxial mesoderm (Oates and Ho, 2002), indicating that cyclic gene function and, by inference, the oscillator, is required for, and restricted to, the positioning of the segmental boundaries only.

By definition (Palmeirim et al., 1997), a *component* of the segmentation oscillator is required for some aspect of oscillator integrity, whereas an *output* is a means of translating the periodicity of the oscillator into an effect in the embryo that does not feed back into the oscillatory mechanism. In operational terms, the loss of a component's function would be expected to disrupt the wavelike expression domains of cyclic genes, but the loss of an output would not, although it may nevertheless result in somitogenic defects. Using these criteria, the cyclic *her7*, *her1*, and *Hes7* genes of zebrafish and mouse are components of the oscillator (Bessho et al., 2001b, 2003; Henry et al., 2002; Holley et al., 2002; Oates and Ho, 2002), whereas the *Hes1* gene of mice appears to be an output (Ishibashi et al., 1995; Jensen et al., 2000; Jouve et al., 2000). Removing either *her7* or both *her7* and *her1* function in zebrafish results in a loss of coordinated oscillatory expression of the cyclic genes; instead, they are expressed evenly throughout the PSM (Gajewski et al., 2003; Henry et al., 2002; Oates and Ho, 2002). Similarly, loss of *Hes7* in the mouse results in the loss of coordinated oscillations and widespread expression of *Lfng* (Bessho et al., 2001b, 2003; Hirata et al., 2004), suggesting that the *HER* genes together normally function in a repressive capacity within the oscillator. Indeed, mathematical modeling of a *Her*-driven negative feedback loop suggests that this interaction, combined with adequate delay, is sufficient for oscillation (Lewis, 2003), although recent data suggest that *her1* may also be capable of acting as an activator in the non-cyclic anterior PSM (Gajewski et al., 2003).

Loss of function of several non-cyclic members of the Delta/Notch signaling system also gives rise to somitogenic defects during mouse and zebrafish embryogenesis, including lesions in *deltaD*, *Dll1*, *Dll3*, *mind bomb*, *notch1a*, *Notch1*, *Presenilin1*, and *Su(H)* (Conlon et al., 1995; Holley et al., 2000, 2002; Hrabe de Angelis et al., 1997; Itoh et al., 2003; Koizumi et al., 2001; Kusumi et al., 1998; Oka et al., 1995; Sieger et al., 2003). Although their mRNAs do not cycle in the PSM, it is possible that one or more properties of their proteins, such as post-translational modification, or the embryonic or sub-cellular distribution, may possess periodic character. Importantly, mutations in the *deltaD*, *Dll1*, *Dll3*, *notch1a*, and *Su(H)* genes result in perturbation of cyclic gene expression (Barrantes et al., 1999; Dunwoodie et al., 2002; Holley et al., 2000, 2002; Jouve et al., 2000; Morales et al., 2002; Sieger et al., 2003), suggesting that they may also be thought of as components of the oscillator.

Loss of function of both cyclic and non-cyclic Delta/Notch and *Her* genes preferentially affects segmentation in the more posterior parts of the animal. In principle, this may be the result of independent mechanisms regulating anterior and posterior trunk segmentation. Alternatively, there may be a single mechanism operating along the A/P axis that requires multiple lesions to be inactivated due to component redundancy. In zebrafish embryos, the onset of defective somitogenesis is sudden, and the position along the A/P axis where the first defective segment occurs, known as the Anterior Limit of Defects (ALD; Oates and Ho, 2002), is characteristic for each mutant or morpholino-induced phenotype. Examination of the wavelike expression domains in these different mutant and morpholino-injected embryos reveals an important relationship between the coherence of cyclic expression patterns and ALD: cyclic patterns are initially normal in the PSM, but gradually lose coherence and at the position of the ALD, have lost any sign of wavelike organization (Jiang et al., 2000; Oates and Ho, 2002).

When combined with a reduction in function of the cyclic *her7* gene, the Delta/Notch loss-of-function mutants *after eight/deltaD* and *deadly seven/notch1a* show a rostral shift in ALD (Oates and Ho, 2002), indicating that these