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beamter/*deltaC* and the role of Notch ligands in the zebrafish somite segmentation, hindbrain neurogenesis and hypochord differentiation

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Abstract

The Tübingen large-scale zebrafish genetic screen completed in 1996 identified a set of five genes required for orderly somite segmentation. Four of them have been molecularly identified and three were found to code for components of the Notch pathway, which are required for the coordinated oscillation of gene expression, known as the segmentation clock, in the presomitic mesoderm (PSM). Here, we show that the final member of the group, *beamter* (*bea*), codes for the Notch ligand DeltaC, and we present and characterize two new alleles, including one allele encoding for a protein truncated in the 7th EGF repeat and an allele deleting only the DSL domain which was previously shown to be necessary for ligand function. Interestingly however, when we over-express any of the mutant *deltaC* mRNAs, we observe antimorphic effects on both hindbrain neurogenesis and hypochord formation. Expression of *bea/deltaC* oscillates in the PSM, and a triple fluorescent in situ analysis of its oscillation in relation to that of other oscillating genes in the PSM reveals differences in subcellular localization of the oscillating mRNAs in individual cells in different oscillation phases. Mutations in *aei/deltaD* and *bea/deltaC* differ in the way they disrupt the oscillating expression of *her1* and *deltaC*. Furthermore, we find that the double mutants have significantly stronger defects in hypochord formation but not in somitogenesis or hindbrain neurogenesis, indicating genetically that the two *delta's* may function either semi-redundantly or distinctly, depending upon context.
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Introduction

DeltaC is one of four known zebrafish members of the Delta subfamily of Notch ligands, all of them transmembrane proteins. Binding of a Delta family member on one cell to the transmembrane receptor Notch on another causes the intracellular domain of Notch to be proteolytically cleaved. This allows the transport of the intracellular fragment N^{ICD} to the nucleus where, in conjunction with the Suppressor of Hairless/RPB-Jκ DNA binding protein, it activates the transcription of target genes including members of the *hairy*/*enhancer of split* family, coding for bHLH transcriptional repressors (Artavanis-Tsakonas et al., 1999; Greenwald, 1998).

Experiments in mouse, zebrafish and *Xenopus* have demonstrated that Notch signaling is essential for the correct formation of somites, the segmented precursors of the vertebral column and skeletal muscle (Bessho et al., 2001; Conlon et al., 1995; del Barco Barrantes et al., 1999; Dornseifer et al., 1997; Dunwoodie et al., 2002; Evrard et al., 1998; Holley et al., 2000, 2002; Hrabé Angelis et al., 1997; Jen et al., 1997; Jen et al., 1999; Jouve et al., 2000; Kusumi et al., 1998; Oka et al., 1995; Takke and Campos-Ortega, 1999; Wong et al., 1997; Zhang and Gridley, 1998). Theories differ, however, as to the exact nature of the role that Notch signaling plays in this process (Giudicelli and Lewis, 2004). Segmentation of the paraxial mesoderm is thought to be regulated by a segmentation clock or oscillator (Cooke, 1998; Cooke and Zeeman, 1975; Meinhardt, 1982, 1986; Palmeirim et al., 1997) and (reviewed in Holley and Takeda, 2002; Pourquié, 2003; Rida et al., 2004; Weinmaster and Kintner, 2003). Somite formation is presaged by stripes of gene expression that appear within the morphologically unsegmented presomitic mesoderm (PSM). Formation of this striped prepattern depends on the segmentation oscillator that operates in the cells of the PSM, causing them to go through repeated cycles of expression and repression of genes associated with the Notch signaling pathway. The oscillation slows down towards the anterior end of the PSM, giving rise to stripes of cells in different phases, visible as spatial waves of gene expression that appear to propagate through the PSM from posterior to anterior. In the zebrafish, *deltaC* is one of the oscillating genes, and in situ hybridization with a *deltaC* probe has been used to demonstrate its oscillation and to show how it is disrupted in various mutants (Holley et al., 2002; Jiang et al., 2000). Other oscillating genes include several *hairy*/*enhancer of split*-related transcription factors in the chick, mouse and zebrafish (Bessho et al., 2001; Gajewski et al., 2003; Holley et al., 2000; Jouve et al., 2000; Leimeister et al., 2000; Oates and Ho, 2002; Palmeirim et al., 1997; Sawada et al., 2000), *lunatic fringe* (*Lfng*) in the mouse and chick (Aulehla and Johnson, 1999; Forsberg et al., 1998; McGrew et al., 1998), and *Axin2* in the mouse (Aulehla et al., 2003). In the zebrafish, these stripes travel roughly one cell diameter every 5–6 min (Holley et al., 2000). At the anterior end of the PSM, the oscillations stop and the pattern is stabilized. The moving boundary between the PSM, where oscillation occurs, and the tissue anterior to it, where oscillation is arrested and morphological segmentation begins, is called the "wave-front" (Cooke, 1998; Cooke and Zeeman, 1975).

The underlying oscillator mechanism is only beginning to be understood (reviewed in Giudicelli and Lewis, 2004). Many observations suggest that the oscillator is based on feedback loops involving the Notch signaling pathway and a number of *hairy/E(spl)*-related transcription factor genes such as *her1* and *her7* in zebrafish and *Hes7* in mouse, which are targets of the Notch pathway (Bessho et al., 2001, 2003; Gajewski et al., 2003; Hirata et al., 2004; Holley et al., 2002; Lewis, 2003; Oates and Ho, 2002). The Notch modulator Lfng is also involved in the mouse and chick (Dale et al., 2003; Serth et al., 2003). In the mouse, however, there is also evidence that a Wnt-dependent clock may act upstream of Notch (Aulehla and Johnson, 1999; Hirata et al., 2004). The wave-front that governs arrest of the oscillation and stabilization of the oscillating prepattern is thought to be specified by a gradient of Fgf signaling, which is highest in the posterior PSM (Dubrulle et al., 2001; Dubrulle and Pourquié, 2004; Sawada et al., 2001). The decline of Fgf signaling below a certain threshold defines the anterior boundary of the PSM, switching on expression of *fss*/*tbx24* (in zebrafish) as the temporal oscillation becomes arrested (Holley et al., 2000; Nikaido et al., 2002). This process results in the segmental expression of a number of genes in the somitic tissue as it emerges at the anterior end of the PSM, including *mesp* genes, Notch pathway genes and genes coding for Eph receptors and ephrins. These genes are thought to collaborate to establish the morphological somite borders via local cell signaling, cell sorting, cell polarization and extracellular matrix assembly (Barrios et al., 2003; Durbin et al., 1998, 2000; Henry et al., 2000; Hrabé Angelis et al., 1997; Jülich et al., 2005; Kim et al., 2000; Koshida et al., 2005; Kulesa and Fraser, 2002; Nakaya et al., 2004; Saga et al., 1997; Sato et al., 2002; Sawada et al., 2000; Takahashi et al., 2000; Topczewska et al., 2001).

It is one thing to show that expression of a gene oscillates, and another to show that it is an essential part of the underlying mechanism that generates oscillations. In the zebrafish, genes coding for essential components of the oscillator mechanism have been identified through a screen for mutations that disrupt the regular periodic pattern of somite segmentation, conducted as part of the large-scale genetic screens published in 1996 (Jiang et al., 1996; van Eeden et al., 1996). Five genes essential for somite segmentation were found: *fused somites* (*fss*), *after eight* (*aei*), *deadly seven* (*des*), *mind bomb* (*mib*, also known as *white tail*), and *beamter* (*bea*). Subsequent work revealed the molecular identity of four of these genes: *aei* was found to code for the Notch ligand DeltaD, *des* for Notch1a, *mib* for an E3 ubiquitin ligase that acts on Delta proteins and is

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