Developmental Biology 327 (2009) 313–326

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

# EphA4 and EfnB2a maintain rhombomere coherence by independently regulating intercalation of progenitor cells in the zebrafish neural keel

Hilary A. Kemp, Julie E. Cooke [1], Cecilia B. Moens *

*Howard Hughes Medical Institute and Division of Basic Science, Fred Hutchinson Cancer Research Center, B2-152, 1100 Fairview Ave. N., Seattle WA 98109, P.O. Box 19024, USA*

ARTICLE INFO

*Article history:*
Received for publication 14 August 2008
Revised 6 December 2008
Accepted 10 December 2008
Available online 24 December 2008

*Keywords:*
Eph
Ephrin
Efn
Hindbrain
Boundary
Zebrafish
Rhombomere
Neuroepithelium
Cell affinity
Cell sorting

ABSTRACT

During vertebrate development, the hindbrain is transiently segmented into 7 distinct rhombomeres (r). Hindbrain segmentation takes place within the context of the complex morphogenesis required for neurulation, which in zebrafish involves a characteristic cross-midline division that distributes progenitor cells bilaterally in the forming neural tube. The Eph receptor tyrosine kinase EphA4 and the membrane-bound Ephrin (Efn) ligand EfnB2a, which are expressed in complementary segments in the early hindbrain, are required for rhombomere boundary formation. We showed previously that EphA4 promotes cell–cell affinity within r3 and r5, and proposed that preferential adhesion within rhombomeres contributes to boundary formation. Here we show that EfnB2a is similarly required in r4 for normal cell affinity and that EphA4 and EfnB2a regulate cell affinity independently within their respective rhombomeres. Live imaging of cell sorting in mosaic embryos shows that both proteins function during cross-midline cell divisions in the hindbrain neural keel. Consistent with this, mosaic EfnB2a over-expression causes widespread cell sorting and disrupts hindbrain organization, but only if induced at or before neural keel stage. We propose a model in which Eph and Efn-dependent cell affinity within rhombomeres serve to maintain rhombomere organization during the potentially disruptive process of teleost neurulation.



## Introduction

The ability of cells to make adhesive contacts with appropriate neighbours, while avoiding others, is a fundamental process that underlies development as well as adult function in multicellular animals, and relies on cell surface determinants that allow cells to identify one another. In the vertebrate hindbrain, which is divided into seven segments or rhombomeres, experimental juxtaposition of cells with different segment identities results in their robust sorting-out (Cooke et al., 2005; Guthrie and Lumsden, 1991; Guthrie et al., 1993; Moens et al., 1996; Waskiewicz et al., 2002; Xu et al., 1999). These cell behaviours are thought to underlie the establishment and maintenance of rhombomere boundaries during normal neuroepithelial development.

Mechanistically, cell sorting has been shown to be driven efficiently by either repulsion between unlike cells or differential adhesion between like cells. The Eph receptor tyrosine kinases and their Ephrin (Efn) ligands are membrane proteins that mediate cell–cell repulsion and attraction in many developmental contexts (Egea and Klein, 2007; Hirashima and Suda, 2006; Klein, 2004; Poliakov et al., 2004; Sela-Donenfeld and Wilkinson, 2005). The outcome of a given Eph–Ephrin interaction is context-dependent, and both in vivo and in vitro, receptor–ligand pairs have been shown to mediate cell–cell repulsion in some instances, while promoting adhesion in others (Eberhart et al., 2004; Hindges et al., 2002; McLaughlin et al., 2003; Santiago and Erickson, 2002).

In the developing zebrafish hindbrain, EphA4 is expressed in rhombomeres 3 and 5 while EfnB ligands EfnB2a and EfnB3 are expressed in the adjacent rhombomeres (Bergemann et al., 1995; Chan et al., 2001; Nieto et al., 1992). Knock-down of EphA4 results in the loss of rhombomere boundaries and the disruption of normal segmental hindbrain neuroanatomy, a phenotype that is exacerbated by simultaneous depletion of EfnB2a, consistent with a critical role for these molecules in regulating the cell sorting behaviours that drive rhombomere boundary formation and maintenance (Cooke et al., 2005). Initial over-expression experiments in which EphA4-over-expressing cells sorted out from EfnB2a-expressing rhombomeres and vice versa suggested that the underlying cell sorting mechanism was mutual cell repulsion driven by Eph–Ephrin interactions at boundaries (Mellitzer et al., 1999; Xu et al., 1999). However by mosaic analysis using loss-of-function reagents we discovered that cells lacking EphA4 sort out from cells expressing EphA4 in r3 and r5 where EfnB2a is not expressed, suggesting that the EphA4 protein has an EfnB2a-independent adhesive role within the forming r3 and r5 territories

* Corresponding author. Division of Basic Science, Fred Hutchinson Cancer Research Center, B2-152, 1100 Fairview Ave. N., Seattle WA 98109, P.O. Box 19024, USA.
*E-mail address:* cmoens@fhcrc.org (C.B. Moens).
[1] Current address: Summit plc, 91 Milton Park, Abingdon, Oxfordshire, OX14 4RY, UK.

0012-1606/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.12.010

(Cooke et al., 2005). Here we describe a corresponding EphA4-independent requirement for the EfnB2a ligand in regulating cell affinity between cells within rhombomere 4 (r4).

Sharpening of rhombomere boundaries in teleosts coincides with the complex morphogenetic movements required for neurulation. Previously we have shown that EphA4-based sorting in mosaic embryos begins in the neural keel (Cooke et al., 2005), an intermediate stage in neurulation characterized by cross-midline cell divisions that generate bilateral clones (Kimmel et al., 1994; Papan and Campos-Ortega, 1999). In this work, we identify the cellular behaviors regulated by EphA4 and EfnB2a in the context of this morphogenesis. Using time-lapse analysis of cell sorting in mosaic embryos, we find that EphA4 and EfnB2a are specifically and individually required to facilitate normal integration of newborn progenitor cells during the cross-midline cell division that occurs at the neural keel/rod stage in zebrafish neurulation. We propose a model in which both EphA4 and EfnB2a are required for rhombomere-specific cell affinity and thus to maintain rhombomere coherence and bilateral symmetry during the

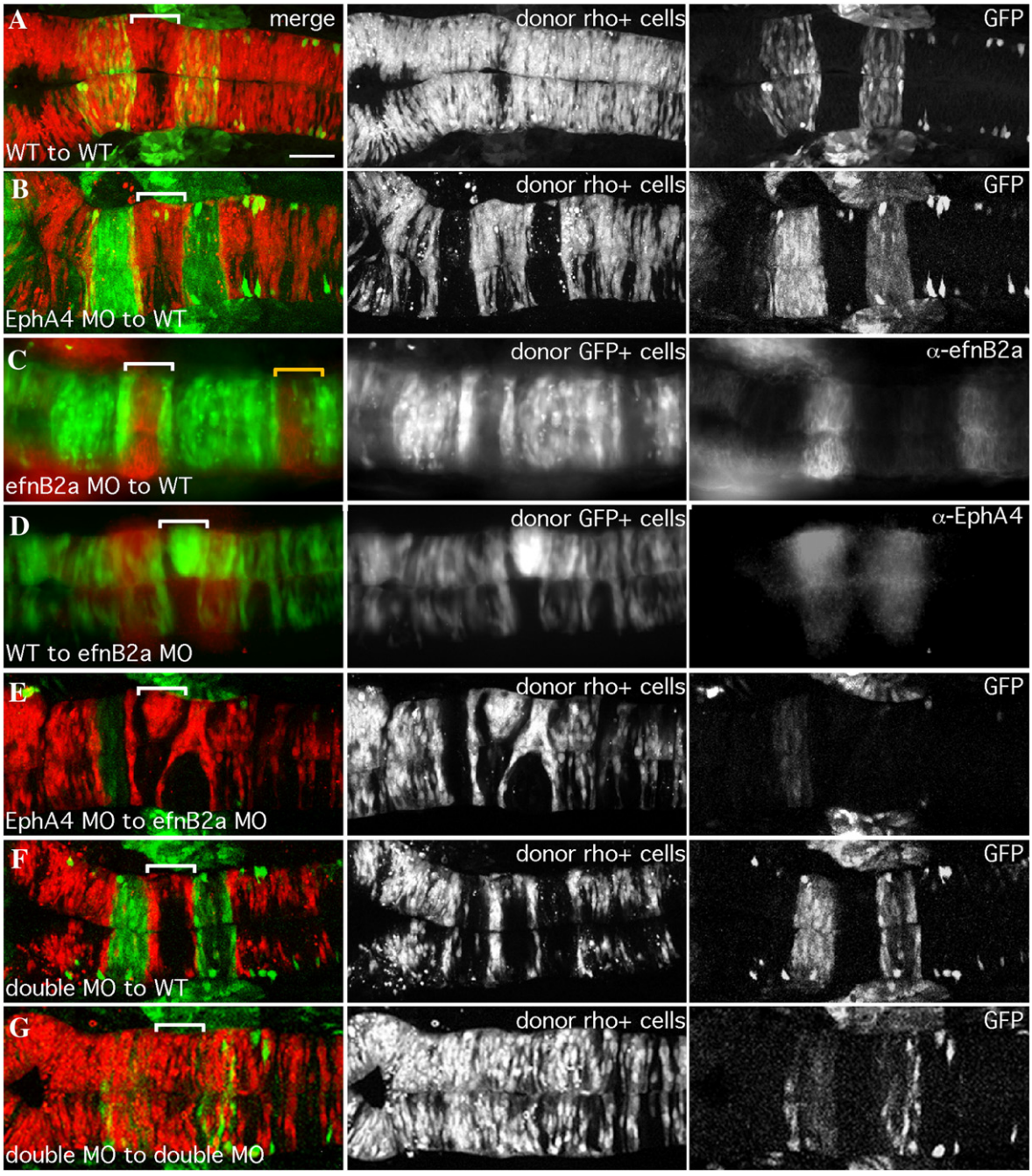


**Fig. 1.** EfnB2a promotes cell adhesion in r4 independent of EphA4. 18 hpf mosaic embryos shown in dorsal view with anterior to the left. Left panels are merged images of individual channels shown in the middle and right panels. Donor cells (middle panel), are labeled with rhodamine dye (red in the merge; A, B, E, F, G) or express GFP (green in the merge; C, D). Rhombomere-specific markers (right panel): r3 and r5 are identified by expression of GFP in pGFP5.3 transgenic hosts (green in the merge; A, B, E, F, G) or detected by α-EphA4 (red in the merge; D); α-EfnB2a marks r1, r4, r7 (red in the merge; C). r4 is indicated by a white bracket in the merge. (A) WT cells contribute evenly to a WT host hindbrain. (B) EphA4 MO donor cells are excluded from r3 and r5 of a WT host. (C) EfnB2a MO donor cells are excluded from r4 and r7 (yellow bracket) of a WT host embryo. (D) WT donor cells form unilateral clusters on one side of r4 of an EfnB2a MO host. (E) EphA4 MO donor cells are excluded from r3 and r5 and form a unilateral cluster in r4 of an EfnB2a MO host embryo. (F) EfnB2a; EphA4 double MO donor cells are excluded from r3, r4, and r5 of WT host embryos. (G) EphA4; EfnB2a double MO donor cells contribute homogeneously to the hindbrain of a double MO host embryo. Scale bar: 50 μm.