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Essential role for Csk upstream of Fyn and Yes in zebrafish gastrulation

Chris Jopling, Jeroen den Hertog *

Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

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Abstract

Morphogenetic cell movements during gastrulation shape the vertebrate embryo bodyplan. Non-canonical Wnt signaling has been established to regulate convergence and extension cell movements that mediate anterior-posterior axis elongation. In recent years, many other factors have been implicated in the process by modulation of non-canonical Wnt signaling or by different, unknown mechanisms. We have found that the Src family kinases, Fyn and Yes, are required for normal convergence and extension cell movements in zebrafish embryonic development and they signal in parallel to non-canonical Wnts, eventually converging on a common downstream factor, RhoA. Here, we report that Csk, a negative regulator of Src family kinases has a role in gastrulation cell movements as well. Csk knock down induced a phenotype that was similar to the defects observed after knock down of Fyn and Yes, in that gastrulation cell movements were impaired, without affecting cell fate. The Csk knock down phenotype was rescued by simultaneous partial knock down of Fyn and Yes. We conclude that Csk acts upstream of Fyn and Yes to control vertebrate gastrulation cell movements.

Keywords: Gastrulation; Cell movements; Csk; Src family kinases; Zebrafish

1. Introduction

A series of morphogenetic cell movements during gastrulation results in the formation of the three germ layers, endoderm, mesoderm and ectoderm and in so doing creates the basic bodyplan of the developing embryo (Warga and Kimmel, 1990). Convergence and extension (CE) represents one of this series of movements during which cells converge towards the midline of the developing embryo, forming the medial/lateral axis, where they intercalate with one another and so extend around the yolk giving rise to the anterior/posterior axis (Keller et al., 1992). In vertebrates this process is governed primarily by the non-canonical Wnt pathway which is similar to the planar cell polarity (PCP) pathway identified in *Drosophila* (Solnica-Krezel and Eaton, 2003).

The vertebrate non-canonical Wnt pathway becomes activated when Wnt11 or Wnt5 bind to Frizzled receptors resulting in the translocation of Dishevelled to the plasma membrane where it forms a complex with Daam1, RhoA and Rac. RhoA and Rac subsequently become activated and propagate the signal to their respective downstream effectors, including Rok and JNK (Habas et al., 2001, 2003; Veeman et al., 2003). In *C. elegans*, this cascade will remodel the cell establishing polarity and allowing it to mount a proper chemotactic response (Goldstein et al., 2006). Non-canonical Wnt signaling induced cell polarization may be at the basis of vertebrate CE cell movements as well. In zebrafish, a number of mutants have been identified that harbor mutations in genes regulating this process (Heisenberg et al., 2000; Sepich et al., 2000; Topczewski et al., 2001; Kilian et al., 2003). The phenotype that all of these mutants have in common is that the embryos are shorter and broader as one might expect if CE has been disrupted. More recently a number of studies have come to light that show that CE is not solely governed by the non-canonical Wnt pathway. Other factors involved include Gα12/13 (Lin et al., 2005), Has2 (Bakkers et al., 2004), Cyclooxygenase-1 (Cha et al., 2005), Widerborst (Hannus et al., 2002), ERRα (Bardet et al., 2005),

* Corresponding author. Tel.: +31 30 2121800; fax: +31 30 2516464.
E-mail address: hertog@niob.knaw.nl (J. den Hertog).

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Scribble-1 (Wada et al., 2005), Fyn and Yes (Jopling and den Hertog, 2005), Ephrins (Oates et al., 1999), Slit (Yeo et al., 2001) and Stat3 (Yamashita et al., 2002). These serve to either modulate non-canonical Wnt signaling directly, highlighted by the recent finding that scribble-1 is a key CE regulator that genetically interacts with trilobite, a known component of non-canonical Wnt signaling (Wada et al., 2005). Alternatively, they function independently of it as shown with *widerborst*, which is not necessary for the activation of non-canonical Wnt signaling but is essential for the correct cellular localization of some of its components (Hannus et al., 2002). Recently, we have shown that signaling through the Src family kinases (SFK) Fyn and Yes converges with non-canonical Wnt signaling and serves to modulate the activity of the small GTPase RhoA during CE cell movements (Jopling and den Hertog, 2005). Furthermore, we have shown that the protein-tyrosine phosphatase (PTP) Shp2, an indirect activator of SFKs (Zhang et al., 2004), is also involved in regulating CE during gastrulation via Fyn/Yes and RhoA (CJ and JdH, unpublished data). Csk antagonizes Shp2 and inhibits SFKs by phosphorylation of a regulatory tyrosine in their COOH-terminus, rendering SFKs inactive (Nada et al., 1991). Therefore, we asked the question ''Is Csk involved in CE during vertebrate gastrulation?''.

Csk knockout mice die prenatally with a complex range of phenotypes including neural tube defects all of which are consistent with defective cell movements during gastrulation (Nada et al., 1993). Mouse knockouts such as *looptail* and *scribble* also display neural tube defects (Murdoch et al., 2001; Murdoch et al., 2003) while their zebrafish homologs, *trilobite* and *scribble-1*, respectively, have been linked directly to the regulation of non-canonical Wnt signaling and show disrupted CE movements during gastrulation (Sepich et al., 2000; Wada et al., 2005). Moreover, loss of Van gogh-like 2 in zebrafish *trilobite* mutants induced neural tube defects as well (Ciruna et al., 2006). Cultured fibroblast cells deficient for *csk* fail to migrate properly in response to various stimuli such as the growth factors PDGF and EGF, a defect that can be rescued by the inhibition of SFKs (McGarrigle et al., 2006). These cells show defective actin cytoskeletal remodelling, which is also observed in *Drosophila* wing cells with defective non-canonical Wnt signaling (Shimada et al., 2001) and in tissue culture cells with impaired non-canonical Wnt signaling (Wechezak and Coan, 2005; Aspenstrom et al., 2006). In *Xenopus*, overexpression of *csk* mRNA results in defective gastrulation cell movements, mimicking the phenotype caused by expression of dominant negative SFKs (Denoyelle et al., 2001).

Here we show that morpholino mediated knockdown of Csk in zebrafish results in defective morphogenetic cell movements during gastrulation without affecting overall cell fate, similar to the phenotype observed when positive regulators such as Fyn and Yes are knocked down. We also show that Csk exerts its effects through the negative regulation of Fyn and Yes.

2. Results

Zebrafish *csk* was identified (EST clone IMAGp998P2017182Q1) based on protein sequence homology with its human and mouse counterparts (86% and 85.6% identical, respectively) (Fig. 1A). *In situ* hybridization experiments using a *csk*-specific antisense probe show that it was ubiquitously expressed throughout early

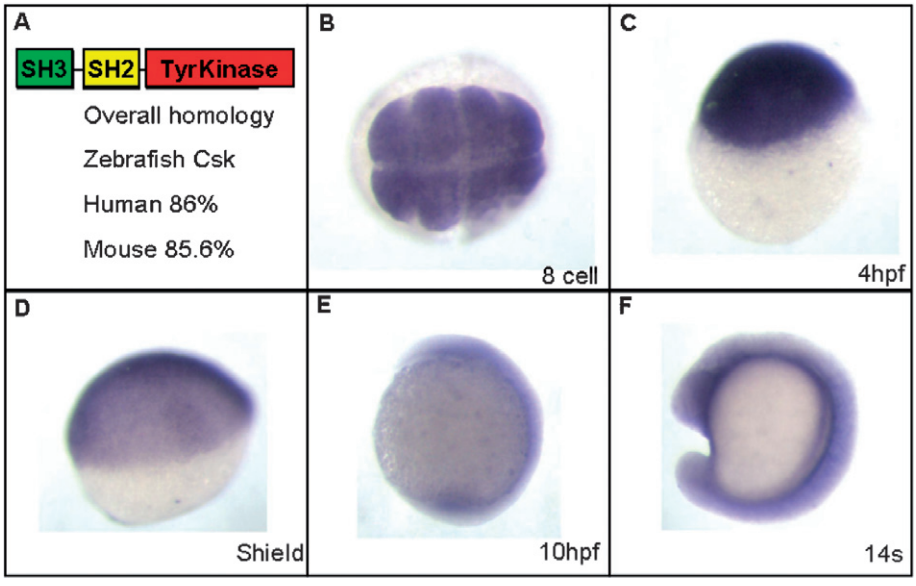

Fig. 1. Csk is ubiquitously expressed in early zebrafish development. (A) Schematic representation of zebrafish Csk with one Src homology 3 (SH3) domain and one Src homology 2 domain to the N-terminal side of the protein-tyrosine kinase domain. The overall sequence identity with human and mouse Csk is indicated. (B–F) *In situ* hybridization with a Csk-specific antisense probe at various stages of development: (B) 8 cell-stage; (C) 4 hpf; (D) shield stage; (E) 10 hpf and (F) 14 somite (14 s).