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Xenopus p21-activated kinase 5 regulates blastomeres adhesive properties during convergent extension movements

Sandrine Faure[a], Julien Cau[a,1], Pascal de Santa Barbara[b], Stéphanie Bigou[a], Qingyuan Ge[c], Claude Delsert[a,d], Nathalie Morin[a,*]

[a]*Centre de Recherches en Biochimie Macromoléculaire, FRE 2593 CNRS, 1919 Route de Mende, 34293 Montpellier, France*
[b]*Institut de Génétique Humaine, UPR 1142 CNRS, 34396 Montpellier, France*
[c]*Cell Signaling Technology, Inc., Beverly, MA 01915, USA*
[d]*IFREMER, 17390 La Tremblade, France*

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Abstract

The p21-activated kinase (PAK) proteins regulate many cellular events including cell cycle progression, cell death and survival, and cytoskeleton rearrangements. We previously identified X-PAK5 that binds the actin and microtubule networks, and could potentially regulate their coordinated dynamics during cell motility. In this study, we investigated the functional importance of this kinase during gastrulation in *Xenopus*. X-PAK5 is mainly expressed in regions of the embryo that undergo extensive cell movements during gastrula such as the animal hemisphere and the marginal zone. Expression of a kinase-dead mutant inhibits convergent extension movements in whole embryos and in activin-treated animal cap by modifying behavior of cells. This phenotype is rescued in embryo by adding back X-PAK5 catalytic activity. The active kinase decreases cell adhesiveness when expressed in animal hemisphere and inhibits the calcium-dependent reassociation of cells, while dead X-PAK5 kinase localizes to cell–cell junctions and increases cell adhesion. In addition, endogenous X-PAK5 colocalizes with adherens junction proteins and its activity is regulated by extracellular calcium. Taken together, our results suggest that X-PAK5 regulates convergent extension movements in vivo by modulating the calcium-mediated cell–cell adhesion.

Keywords: X-PAK5; *Xenopus* embryogenesis; Cell adhesion; Convergent extension

Introduction

During gastrulation, germ layers undergo temporally and spatially coordinated movements that bring progenitor cells into positions from which they later form tissues and organs. In *Xenopus*, convergence extension (CE) movements are essential early during development for the formation and the elongation of the body axis. During CE movements, cells of the marginal zone polarize, elongate, and align before they intercalate between one another (Keller et al., 2000; Shih and Keller, 1992). These lateral cell movements result in the mediolateral narrowing (convergence) and anteroposterior lengthening (extension) of the embryo.

In *Xenopus* embryo, Wnt signaling is best known as a regulator of dorsal cell fate through a « canonical » β-catenin pathway. Wnt also tightly regulates CE movements through activation of two « noncanonical » pathways that respond to members of Wnt superfamily ligands such as Wnt-5a and Wnt-11 (Tada and Smith, 2000; Torres et al., 1996). These ligands, respectively, turn on the Wnt/Ca^{2+} and the Wnt/JNK pathways (the vertebrate equivalent to the planar cell polarity or PCP pathway in *Drosophila*) (for review, see Kuhl, 2002; Tada et al., 2002; Wallingford et al., 2002) that act as antagonists of the Wnt/β-catenin signaling (for review, see Weidinger and Moon, 2003). In response to noncanonical Wnt signaling, cells eventually develop the capacity to change their shape, their polarity,

* Corresponding author. Fax: +33 4 67 52 1559.
E-mail address: morin@crbm.cnrs-mop.fr (N. Morin).
[1] Present address: MRC/Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT, UK.

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and their adhesive properties, thus allowing CE movements to occur.

Indeed, the family of Rho GTPases that controls cytoskeleton reorganization and cell adhesion (for review, see Hall and Nobes, 2000; Jaffer and Chernoff, 2004; Kaibuchi et al., 1999; Schmitz et al., 2000) is also essential for regulating CE movements in *Xenopus* embryo. Inactivation of either endogenous Rac, Cdc42, or RhoA prevents CE movements to occur (Choi and Han, 2002; Habas et al., 2001, 2003; Tahinci and Symes, 2003). Both RhoA and Rac are effectors of the PCP pathway (Habas et al., 2001, 2003) although their activation is not dependent upon each other (Habas et al., 2003). Cdc42 is involved in Wnt/Ca^{2+} signaling and regulates CE movements downstream of Gβγ (Penzo-Mendez et al., 2003) and PKC (Choi and Han, 2002; Penzo-Mendez et al., 2003). While clearly GTPases are involved in CE, the GTPase effectors that regulate changes in polarity, protrusive, and adhesive activities of cells during CE are not well characterized. Changes in polarity and directional movements could involve effectors that capture and stabilize microtubules near the cell cortex such as IQGAP1 and Par6 downstream of Cdc42 or mDia downstream of RhoA (Etienne-Manneville and Hall, 2001; Fukata et al., 2002; Palazzo et al., 2001). Changes in adhesive properties, morphology, protrusive activity, and generation of forces for cell translocation and movements involve reorganization of the actin cytoskeleton and the adherence junctions. A number of GTPase effectors downstream of Rac and Cdc42 could be involved. p21-activated kinases (PAKs) are good candidates for this role since they have been shown to control cell shape and motility (for review, see Bokoch, 2003; Jaffer and Chernoff, 2002). PAK subgroups I and II are distinguished based upon their structural organization and regulation. Binding of GTP-bound Rac or Cdc42 to subgroup I PAKs (PAK1–3) causes the dissociation of the PAK kinase autoinhibitory domain (AID) and the C-terminal catalytic domain, thus allowing autophosphorylation and full activation of the kinase (Buchwald et al., 2001; Chong et al., 2001; Lei et al., 2000). In contrast, subgroup II PAKs (PAK4–6) appear to lack a classic AID and are not directly activated by binding of active Rac and Cdc42 GTPases. Both subgroup kinases are involved in the regulation of MAP kinase cascades, cell cycle, and apoptosis (for review, see Bokoch, 2003; Jaffer and Chernoff, 2002), and were shown to regulate cell cytoskeletal changes. Individual PAKs act through a number of targets including the myosin light chain kinase (MLCK) (Sanders et al., 1999), regulatory myosin light chain (Goeckeler et al., 2000), Caldesmon (Foster et al., 2000), filamin (Vadlamudi et al., 2002), desmin (Ohtakara et al., 2000), Lim kinase (Dan et al., 2001; Edwards et al., 1999), and integrin alpha v beta 5 (Zhang et al., 2002). Subgroup I and II PAKs may carry out different functions in a same cell as highlighted in Schneeberger and Raabe (2003). In developing photoreceptor cells, *Drosophila* subgroup II PAK Mbt depends upon Cdc42 binding for its localization to adherence junctions (Schneeberger and Raabe, 2003) and is required for cell morphogenesis, while DPAK, a subgroup I PAK, is required in growth cones to control axon guidance (Hing et al., 1999).

Human PAK4, a member of subgroup II, induces loss of cell adhesion and anchorage-independent growth that characterize oncogenic cell transformation (Callow et al., 2002; Qu et al., 2001). We previously described a subgroup II *Xenopus* PAK, X-PAK5, that is closely related to hPAK4. X-PAK5 binds the actin and microtubule networks (Cau et al., 2001), and binding of active GTPases to the endogenous kinase induces its relocalization to actin-rich structures. Both the actin and microtubule networks are important in the regulation of cell motility. This led us to investigate whether X-PAK5 may regulate the extensive cell rearrangements that occur during gastrulation in *Xenopus*. In this report, we show that X-PAK5, which is expressed during early development, is present in regions where morphogenetic movements are induced at the onset of gastrulation. Expression of kinase-dead mutant impairs CE movements and results in defects in blastopore closure. Kinase-dead mutants act at least by strengthening cell–cell junctions in embryonic cells while active X-PAK5 changes the adhesive properties of cells. Changes in cell adhesion eventually lead to embryos that develop tissue protrusions. Finally, our data indicate that the catalytic activity of X-PAK5 is regulated upon calcium-induced aggregation of dissociated cells. We finally show that endogenous, active, and dead-kinase mutants of X-PAK5 colocalize at least partially with adherens junction proteins.

Materials and methods

Embryo manipulation

Eggs were collected from *Xenopus* females and artificially fertilized as previously described (Watanabe and Whitman, 1999). Embryos were dejellied before the first cleavage in 3% cysteine and injected in 3% Ficoll in 0.1× Marc's modified Ringer medium (MMR). Embryos were staged according to Nieuwkoop and Faber (1967). Animal caps were dissected at stage 8.5 and incubated in 0.7× MMR in the presence or absence of partially purified human activin A at a final concentration of 8 units/ml (Cooke et al., 1987) (kindly provided by J.C. Smith). Animal caps were cultured to stage 18 equivalent. Cycloheximide treatment was performed by transferring embryos to 0.1 mg/ml cycloheximide in 0.7× MMR.

Anti-X-PAK antibodies

The polyclonal anti-X-PAK5 antibodies have been raised against the unique 122–224 amino acids of X-PAK5 (Cau et al., 2001). Immunopurified antibodies specifically detect a single band of 75 kDa in *Xenopus* eggs and embryos.