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Roles for focal adhesion kinase (FAK) in blastomere abscission and vesicle trafficking during cleavage in the sea urchin embryo

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ABSTRACT

Is focal adhesion kinase (FAK) needed for embryonic cleavage? We find that FAK is expressed during early cleavage divisions of sea urchin embryos as determined by polyclonal antibodies to the *Lytechinus variegatus* protein. FAK is absent in eggs and zygotes and then cycles in abundance during the first cleavages after fertilization. It is maximal at anaphase, similar to the destruction and synthesis of cyclin proteins. To investigate whether FAK is needed during early cleavage, we interfered with its function by microinjecting eggs with anti-FAK antibodies or with FAK antisense morpholino oligonucleotides. Both treatments led to regression of the cleavage furrow. FAK knockdown with antibodies or morpholino oligonucleotides also resulted in an over-accumulation of endocytic vesicles. Thus, FAK could be restricting endocytosis or increasing exocytosis in localized areas important for abscission. FAK appears to be necessary for successful cleavage. These results are the first to document a functional role for FAK during embryonic cleavage.

1. Introduction

Cytokinesis requires a dynamic actin cytoskeleton and active remodeling of the plasma membrane. These changes include reorganization of the cortical cytoskeleton, modification of vesicle trafficking that changes the cell surface and extracellular matrix molecules, and partitioning of the membranes between the two cells to produce the daughter blastomeres (Wong et al., 1997; Shuster and Burgess, 2002; Saint and Somers, 2003; Inoue et al., 2004; Albertson et al., 2005; Foe and Von Dassow, 2008; Werner and Glotzer, 2008; Steigemann and Gerlich, 2009; Fededa and Gerlich, 2012). Completion of cytokinesis is actively regulated by specific proteins and proceeds through a series of discrete and essential steps. These steps include: (1) assembly and positioning of the astral spindle (Larkin and Danilchik, 1999, 2001; Strickland et al., 2005; Yüce et al., 2005; D'Avino et al., 2006; Odell and Foe, 2008; von Dassow et al., 2009); (2) constriction of the actomyosin contractile ring to form the cleavage furrow through Rho-family GTPases (Kishi et al., 1993; Jantsch-Plunger et al., 2000;

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Piekny et al., 2005; Chalamalasetty et al., 2006; Bement et al., 2006; Canman et al., 2008), (3) migration of vesicles and the centralspindlin complex on microtubules to the midbody (Danilchik et al., 1998; Ng et al., 2005; Li et al., 2006; Albertson et al., 2008; Simon et al., 2008; McKay and Burgess, 2011), (4) anchoring of endocytic vesicles in late stages of mitosis to the cleavage furrow (Schweitzer et al., 2005; Wilson et al., 2005), and finally (5) fusion of endocytic vesicles or constriction by ESCRT-III to complete abscission of the daughter cells (Rappaport, 1997; Barr and Gruneberg, 2007; Manchinelly et al., 2010; Neto and Gould, 2011; Guizetti et al., 2011; Carlton et al., 2012). Here we investigate relationships between these cytokinesis events and focal adhesion kinase signaling.

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase. It is found in migrating cells that form protein clusters at focal adhesion sites with integrins involved in cell attachment (reviewed by Mitra and Schlaepfer (2006), Tomar and Schlaepfer (2009), Frame et al. (2010) and Schaller (2010)). FAK, a key signaling kinase in these structures, interacts with many proteins including the kinases Src and Cas and the proteins paxillin and talin, which link FAK to the integrins (Yamakita et al., 1999; Chatzizacharias et al., 2008). Signaling through FAK also mediates cell survival, the stabilization of lipid rafts, the stabilization of microtubule interaction with the membrane, endocytosis, vesicle trafficking (Almeida et al., 2000; Guan, 2004; Palazzo et al., 2004; Avizienyte and Frame, 2005; Wu et al., 2005). FAK can be activated independent of integrin signaling and cell adhesion by ezrin-family proteins or by phosphatidylinositol 4,5-bisphosphate (PIP_2) localized to the cleavage furrow (Linseman et al., 1999; Poullet et al., 2001; Field et al., 2005; Janetopoulos et al., 2005; Wo ng et al., 2005; Logan and Mandato, 2006; Cai et al., 2008; Nezis et al., 2010). The integration of multiple stimuli and mediation of downstream signals through an array of protein interactions reveal FAK's importance in diverse cellular processes (Mitra et al., 2005).

Vesicle trafficking is required for abscission to occur (Albertson et al., 2005, 2008; Matheson et al., 2005; Ng et al., 2005; Schweitzer et al., 2005; Barr and Gruneberg, 2007; Echard, 2008). FAK-stimulated Src phosphorylation of endophilin inhibits its dynamin-mediated endocytosis (Wu et al., 2005), whereas FAK-stimulated Src phosphorylation activates endocytosis of cadherins (Avizienyte and Frame, 2005). FAK could be involved in the turnover of vesicles near the cleavage furrow. FAK signaling plays a role in vesicle trafficking, including endocytosis of integrin complexes (Ezratty et al., 2005; Chao et al., 2010; Wang et al., 2011) and exocytosis of vesicles (Doucey et al., 2003; Rosse et al., 2009; Gupton and Gertler, 2010). We have found FAK in the cortex of the zygote and early cleavage stages of the sea urchin embryo (García et al., 2004). Abscission was aborted and endocytic vesicles were misregulated when we knocked down FAK with antibodies or morpholino oligonucleotides. Here we report the first observation of a requirement for FAK during abscission.

2. Results

2.1. FAK oscillates during cleavage

FAK was monitored during early cleavage by immunostaining whole embryos with an affinity-purified polyclonal antibody to the focal adhesion targeting domain (FAT) at the C-terminus of *Lytechinus variegatus* FAK. As shown below, FAK morpholino eliminated most staining of the antibody demonstrating its specificity. FAK was not detected in the fertilized egg (Fig. 1a), but increased in the late zygote (Fig. 1b). During initial formation of the cleavage furrow, FAK localized to the cortex (Fig. 1c, double arrowhead). FAK was seen at the leading edge of the forming furrow but was excluded from the cytoplasm that lies in the plane of the future cleavage site (Fig. 1c, arrowhead). When blastomere abscission appeared complete, FAK was enriched in the entire cortex, including the surface between the two new blastomeres (Fig. 1d, arrow). FAK declined abruptly in the middle of the two-cell stage (Fig. 1e) but then increased in the cytoplasm at the onset of the second cleavage (Fig. 1f). As cleavage proceeded, FAK was again enriched in the cortex of the forming blastomeres (Fig. 1g), and when abscission was complete, immunoreactivity declined again (Fig. 1h). Thus, FAK oscillated consistently through the first cleavages, being localized most strongly to the cell cortex during cleavages, and was present in the forming cleavage furrow.

To verify the stages during which FAK increases and decreases in the cleaving embryos, *Lytechinus pictus* embryos were stained with antibodies to tubulin and with DAPI to stain DNA, as well as with the affinity-purified antibody to sea urchin FAK. Fig. 2A shows the triple stain, as well as the FAK fluorescence alone. At prophase (top panels), FAK fluorescence was low and continued low through early prometaphase. In late prometaphase and as the cells entered early anaphase, FAK fluorescence increased and was very high when the embryos entered late anaphase. But in telophase, the FAK fluorescence significantly declined. Fig. 2B compares the maximum fluorescing regions, which were next to the nucleus, as well as the division plane between the two cleaving cells for the different stages. Cytoplasmic FAK increases beginning in prometaphase and decreases at telophase concomitant with an enrichment of FAK at the cleavage furrow. Thus again, FAK cycles with the cell cycle.

To understand the cellular oscillations of FAK protein, we investigated protein levels with western blots of the embryos in early cleavage stages (Fig. 3). We isolated cell homogenates from synchronized embryos and probed the western blots for both FAK and the housekeeping protein glyceraldehye-3-phosphate-dehydrogenase (GAPDH) (Fig. 3A). FAK was present at low levels in eggs and zygotes for 60 min. It increased during anaphase of the first cleavage, declined during telophase and prophase and increased again in the second cleavage (Fig. 3B and C). The early cyclic behavior of FAK protein parallels the observations in Figs. 1 and 2 that FAK immunostaining rises during cleavage furrow ingression and blastomere formation (Figs. 1 and 2). The cycling behavior suggests that *L. variegatus* and *L. pictus* FAK have a mechanism for cyclic destruction synchronized with cell division.

2.2. FAK knockdown results in regression of the cleavage furrow

We tested the hypothesis that the cycling of FAK is required for the cell cycle by injecting zygotes with the affinity-purified anti-FAK antibody to block the function of FAK (Fig. 4A). As a control, zygotes were injected with