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Regulation of sperm motility by $PIP_{2(4,5)}$ and actin polymerization

Maya Finkelstein, Bar Megnagi, Debby Ickowicz, Haim Breitbart *

The Mina and Everard Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

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ABSTRACT

Actin polymerization and development of hyperactivated (HA) motility are two processes that take place during sperm capacitation. In previous studies, we demonstrated that the increase in F-actin during capacitation depends upon inactivation of the actin severing protein, gelsolin, by its binding to phosphatydilinositol-4, 5-bisphosphate (PIP_2). Here, we showed for the first time the involvement of PIP_2/gelsolin in human sperm motility before and during capacitation. Activation of gelsolin by causing its release from PIP_2 inhibited sperm motility, which could be restored by adding PIP_2 to the cells. Reduction of PIP_2 synthesis inhibited actin polymerization and motility, and increasing PIP_2 synthesis enhanced these activities. Furthermore, sperm demonstrating low motility contained low levels of PIP_2 and F-actin. During capacitation there was an increase in PIP_2 and F-actin levels in the sperm head and a decrease in the tail. In sperm with high motility, gelsolin was mainly localized to the sperm head before capacitation, whereas in low motility sperm, most of the gelsolin was localized to the tail before capacitation and translocated to the head during capacitation. We also showed that phosphorylation of gelsolin on tyrosine-438 depends on its binding to PIP_2. Activation of phospholipase C by Ca^{2+}-ionophore or by activating the epidermal-growth-factor-receptor inhibits tyrosine phosphorylation of gelsolin. In conclusion, the data indicate that the increase of PIP_2 and/or F-actin in the head during capacitation enhances gelsolin translocation to the head. As a result the decrease of gelsolin in the tail allows keeping high level of F-actin in the tail, which is essential for the development of HA motility.

Introduction

Ejaculated spermatozoa must undergo a series of biochemical modifications in order to attain the ability to penetrate and fertilize the oocyte. These processes occur in the female reproductive tract and are collectively called capacitation. The capacitated spermatozoon is able to bind to the Zona Pellucida (ZP) of the oocyte and to undergo the acrosome reaction (AR), a process that allows sperm penetration and fertilization of the egg (reviewed in Yanagimachi (1994)).

Capacitation includes multiple physiological and biochemical modifications (reviewed by (Breitbart, 2003; Breitbart and Etkovitz, 2011; Salicioni et al., 2007; Visconti et al., 1995)). The biochemical changes associated with the capacitation process include an efflux of cholesterol from the plasma membrane leading to an increase in membrane fluidity and permeability to bicarbonate and calcium ions, hyperpolarization of the plasma membrane (Hernandez-Gonzalez et al., 2006), changes in protein phosphorylation and protein kinase activity (Arcelay et al., 2008; Baldi et al., 2000; Visconti, 2009), and increases in bicarbonate concentration (HCO_3^-), intracellular pH (pHi), Ca^{2+} and cAMP levels.

Additionally, our previous studies showed that actin polymerization occurs during sperm capacitation, and rapid depolymerization occurs prior to the acrosome reaction (Breitbart et al. 2005; Brener et al., 2003; Cohen et al., 2004). Actin is present in the sperm head in acrosomal, equatorial and postacrosomal regions as well as in the tail (Clarke et al., 1982; Fouquet and Kann, 1992; Ochs and Wolf, 1985; Virtanen et al., 1984). The presence of actin in the tail might be important for the regulation of sperm motility (Itach et al., 2012), and its presence in the head suggests the possible involvement of actin in the sperm acrosome reaction (Finkelstein et al., 2010).

A connection was reported between actin polymerization and cell motility in other cell types (Bernheim-Groswasser et al., 2005; Carlier et al., 2003; Hotulainen and Lappalainen, 2006). Earlier studies in guinea pig sperm showed that F-actin is involved in sperm motility, and random severing of F-actin filaments inhibits flagellar motility (Azamar et al., 2007).

During the capacitation process, sperms change their motility pattern from progressive to HA motility (de Lamirande and Gagnon, 1993; Goodson et al., 2011). HA motility is characterized by an increase in flagellar bending amplitude, and an increase in average lateral head movement (Demott and Suarez, 1992;

* Corresponding author. Fax: +972 3 5344766.
E-mail address: goldra@biu.ac.il (H. Breitbart).

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Katz and Vanagimachi, 1980; Suarez et al., 1987). It was shown that the efficiency of penetration of hyper-activated sperm to the ZP is much higher than that of non-hyper-activated sperm (Ho and Suarez, 2001). The HA motility may help spermatozoa swim faster and generate enough force to penetrate cumulus cells and ZP during fertilization (Jin et al., 2007). Inhibition of HA motility prevents fertilization from occurring (Amieux and McKnight, 2002). In addition, our recent study indicated the importance of actin polymerization for the development of HA motility. Sperm motility as well as the development of HA motility during capacitation is also mediated by PLD-dependent actin polymerization (Itach et al., 2012). Phosphatidylinositol 4, 5-bisphosphate (PIP_2), the major polyphosphoinositide in mammalian cells, is required as a cofactor for the activation of PLD in many cell types (Brown et al., 1993; Hodgkin et al., 2000; Liscovitch et al., 1994; Pertile et al., 1995; Schmidt et al., 1996).

PIP_2 is a minor lipid of the inner leaflet of the plasma membrane that controls the activity of numerous proteins and serves as a source of second messengers. Aside from the fundamental signaling role of its derivatives, PIP_2 itself controls the activity of several integral membrane proteins, and affects many proteins associating with the membrane due to PIP_2 binding. Although PIP_2 comprises only 1% of all plasma membrane phospholipids, its extraordinary versatility puts it in the center of plasma membrane dynamics governing motility, cell adhesion, endo- and exocytosis (Di Paolo and De Camilli, 2006; Hokin and Hokin, 1953; Yin and Janmey, 2003). Early studies established the role of PIP_2 as the source of two second messengers in the cell, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (Berridge and Irvine, 1984). PIP_2 is important in exocytic and endocytic membrane traffic (Cremona and De Camilli, 2001; Martin, 1998; Ungewickell and Hinrichsen, 2007), ion channel and transporter function (Hilgemann et al., 2001; Suh and Hille, 2005), enzyme activation (McDermott et al., 2004), and protein recruitment (Balla, 1998, 2005; Janmey and Lindberg, 2004; Lemmon, 2003; Takenawa and Itoh, 2006).

PIP_2 serves as an effector of multiple downstream proteins such as MARCKS, gelsolin, PLD, and PI3K. These proteins are present in sperm cells (Etkovitz et al., 2007; Jungnickel et al., 2007) and are involved in regulation of sperm capacitation and/or the acrosome reaction.

Actin polymerization is one of the processes occurring during sperm capacitation, and F-actin breakdown must take place to achieve the acrosome reaction (Brener et al., 2003). The assembly of G-actin to form F-actin, as well as the disassembly of F-actin is highly regulated events (Tanaka et al., 1994; von Bulow et al., 1995, 1997). Gelsolin severs assembled actin filaments, and caps the fast growing plus end of free or newly severed filaments in response to Ca^{2+}, and is inhibited by PIP_2. In a recent study, we showed that the actin severing protein, gelsolin, must be inhibited during capacitation for actin polymerization to occur (Finkelstein et al., 2010).

Functional analysis of fibroblasts isolated from gelsolin knockout mice indicated that gelsolin mediates cell migration, since gelsolin deficiency resulted in the formation of excessive stress fibers, defective ruffling and cell motility (Azuma et al., 1998; Witke et al., 1995). In view of the biological activities of gelsolin, these data suggested that it plays an essential role in the regulation of actin polymerization and cycling necessary for cell motility. A correlation between the level of gelsolin expression, cancer, cell migration and organization of the actin cytoskeleton in other cells was shown, as well (Litwin et al., 2009).

Phosphoinositides bind gelsolin and release it from actin filament ends, exposing sites for actin assembly (Janmey et al., 1987). We showed that the release of bound gelsolin from PIP_2 by PBP10, a peptide containing the PIP_2-binding domain of gelsolin, or by activation of phospholipase C, which hydrolyzes PIP_2, causes rapid Ca^{2+}-dependent F-actin depolymerization as well as an enhanced acrosome reaction (Finkelstein et al., 2010).

Even though the role of gelsolin and actin polymerization is well established, the presence and the possible role of this protein in sperm motility are not fully understood. We therefore hypothesized that PIP_2 and gelsolin are involved in regulating sperm motility and in the development of HA motility in sperm capacitation. In support of our hypothesis, we observed that an increase in PIP_2 and F-actin in the sperm head during capacitation leads to the translocation of gelsolin from the tail to the head, allowing an increase in F-actin in the tail, and resulting in the development of HA motility. We further showed that the basal levels of PIP_2 and F-actin and the localization of gelsolin, control cell motility before capacitation, as well as the development of hyper-activated motility during sperm capacitation.

Materials and methods

Materials

PBP10 (Polyphosphoinositide-Binding Peptide, rhodamine B conjugated), U73122, and A23187 were obtained from Cal-Biochem (San Diego, CA). Capacitation medium, F-10 (HAM) nutrient mixture with L-glutamine, was purchased from Biological Industries (Kibbutz Beit Haemek, IL). SU6656 was obtained from Cal-Biochem. Rabbit polyclonal anti-gelsolin and rabbit polyclonal to EGFR (y845) were obtained from Abcam; anti-beta-actin HRP-conjugated and secondary goat anti-rabbit IgG (H+L)–HRP conjugate were obtained from Bio-Rad (Bio-Rad Lab., Richmond, CA, USA). Secondary mouse anti-rabbit Alexa-fluor-568 IgM and anti-PIP_2 (sc-53412) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Alexa-fluor-488 goat anti-rabbit was obtained from Invitrogen (Oregon, USA). Antibody against P-gelsolin Tyr438 was kindly provided by Dr. Joël Vandekerckhove from the Department of Biochemistry, Ghent University (UGent) (Department of Medical Protein Research, Flanders Institute for Biotechnology (VIB) Albert Baertsoenkaai 3B-9000 Gent, Belgium). All other chemicals were purchased from Sigma (Sigma-Aldrich Israel Ltd., Rehovot, Israel) unless otherwise stated.

Sperm preparation

Human semen was liquefied; afterward, the semen was loaded on a gradient (PureCeptyion Lower and Apper Phase Gradient 80% and 40%) and centrifuged for 30 min at 6750 rpm at room temperature. The lower layer containing the sperm was collected and washed twice in HAM F-10, then spun again and allowed to "swim up" after the last wash at 37 °C. The motile cells were collected without the pellet and resuspended in capacitation medium.

Sperm samples were separated to "high" ($>55\%$ motile cells) and "low" ($<40\%$ but $>20\%$ motile cells) motilities according to the preliminary parameters provided by male fertility laboratory of Sheba hospital.

Sperm capacitation

Human sperm (1×10^7 cells/ml) was capacitated by incubation in capacitation media, HAM F-10 supplemented with 3 mg/ml BSA. The cells were incubated in this medium for 3 h at 37 °C in 5% CO_2. The capacitation state of the sperm was confirmed after the 3 h incubation by examining the ability of the sperm to undergo the acrosome reaction.