Regulation of sperm capacitation and the acrosome reaction by PIP$_2$ and actin modulation

Haim Breitbart, Maya Finkelstein

Asian Journal of Andrology (2015) 17, 597–600; doi: 10.4103/1008-682X.154305; published online: 11 May 2015

Actin polymerization and development of hyperactivated (HA) motility are two processes that take place during sperm capacitation. Actin polymerization occurs during capacitation and prior to the acrosome reaction, fast F-actin breakdown takes place. The increase in F-actin during capacitation depends upon inactivation of the actin severing protein, gelsolin, by its binding to phosphatidylinositol-4, 5-bisphosphate (PIP$_2$) and its phosphorylation on tyrosine-438 by Src. Activation of gelsolin following its release from PIP$_2$ is known to cause F-actin breakdown and inhibition of sperm motility, which can be restored by adding PIP$_2$ to the cells. Reduction of PIP$_2$ synthesis inhibits actin polymerization and motility, while increasing PIP$_2$ synthesis enhances 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 spermatozoa 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 upon its binding to PIP$_2$. Stimulation of phospholipase C$_y$ by Ca$^{2+}$-ionophore or by activating the epidermal-growth-factor-receptor, inhibits tyrosine phosphorylation of gelsolin and enhances enzyme activity. In conclusion, these 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 the maintenance of high levels of F-actin in this structure, which is essential for the development of HA motility.

To acquire the ability to fertilize the egg, mammalian spermatozoa must undergo several biochemical and morphological changes in the female reproductive tract, collectively called capacitation. These changes include cAMP/PKA activation, cholesterol efflux from the plasma membrane, PKA-dependent protein tyrosine phosphorylation, actin polymerization and the development of HA motility. In a recent study we suggested a mechanism by which the Ser/Thr targeting proteins such as Myristoylated alanine-rich C-kinase substrate (MARCKS), gelsolin, PLD and PI3K. These proteins are present in spermatozoa and are involved in the regulation of sperm capacitation and/or the acrosome reaction. PIP$_2$ binds gelsolin and release it from actin filaments ends, exposing sites for actin assembly. We have shown that the release of bound gelsolin from PIP$_2$ causes rapid Ca$^{2+}$-dependent F-actin depolymerization as well as an increased acrosome reaction. We have also shown that PIP$_2$, gelsolin and sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) are involved in regulating sperm motility and the development of HA motility.

LOCALIZATION OF ACTIN AND GELSONIN BEFORE AND AFTER CAPACITATION

It is shown in Figure 1 and Table 1 that the cellular level of F-actin in human spermatozoa is relatively low before capacitation but is significantly enhanced during capacitation mainly in the sperm head but also in the tail. Prior to the acrosome reaction (AR) fast dispersion of F-actin occurs and this process allows the outer acrosomal membrane and the overlying plasma membrane to interact and fuse to culminate in the AR.
Gelsolin, an actin-severing protein, is localized to the G-actin (actin monomers) fraction before capacitation but to the F-actin (filamentous actin) fraction at the end of the capacitation. Moreover, there is a significant increase in Ca^{2+}-dependent gelsolin translocation to the sperm head during capacitation concomitantly with the increase in F-actin.

F-actin is also increased in the sperm tail during capacitation, and is thought to be required for the development of HA motility. Thus it is likely that there are two reasons for the translocation of gelsolin from the tail to the head; first gelsolin is required in the head to depolymerize F-actin to allow the occurrence of the AR and second the exclusion of gelsolin from the tail prevents F-actin depolymerization in the tail allowing the development of HA motility. It is known that PIP_{2} binds gelsolin and we suggest that this binding keeps gelsolin in an inactive/phosphorylated form allowing F-actin formation during capacitation. We found an increase in PIP_{2} in the sperm head during capacitation simultaneously to the increase in F-actin and gelsolin in the head. These findings support our hypothesis that PIP_{2} elevation in the sperm head keeps gelsolin inactive and enables the assembly of F-actin in the head. Interestingly, examination of gelsolin location in ejaculated human sperm with high motility revealed high level of gelsolin in the head, while in human spermatozoa with poor motility, a large proportion of gelsolin was localized in the tail and translocated to the sperm head only after capacitation. Moreover, releasing of gelsolin from binding to PIP_{2} by activation of phospholipase C (PLC) to hydrolyse PIP_{2} or using PBP10, a peptide derived from PIP_{2}-binding domain of gelsolin which competes with gelsolin binding to PIP_{2}, induced the reverse translocation of gelsolin from the head to the tail. Also, inhibition of PIP_{2} synthesis prevented the translocation of gelsolin to the head while enhancing PIP_{2} synthesis significantly increased it. Furthermore, we found that tyr-438 phosphorylation/inhibition of gelsolin is reduced when PIP_{2} levels are decreased and vice versa. These data indicate that the cellular levels of PIP_{2} regulate gelsolin phosphorylation and activation.

**ACTIVATION OF GELSON IN CAPACITATED SPERM**

Gelsolin activation is regulated by calcium ions, phosphoinositides and by Src-dependent phosphorylation on tyr-438. Low concentration of calcium ions cause conformational changes in the C-terminus of gelsolin, which expose its F-actin binding site while higher calcium levels cause a second conformational change exposing the catalytic site. In human spermatozoa, activation of gelsolin by enhancing intracellular calcium concentration or by using the peptide PBP10 causes fast depolymerization of F-actin and induction of the AR in capacitated sperm. In Sertoli cells, the hydrolysis of PIP_{2} by PLC resulted in the release the bound gelsolin and its activation. A scheme describing the physiological activation of PLC is seen in Figure 2. It is well known that activation of EGFR by EGF or elevation of intraspermatozoal [Ca^{2+}] using Ca^{2+}-ionophore activates PLC. In Table 2 we show that EGF or Ca^{2+}-ionophore induces fast F-actin depolymerization and the AR while both are blocked by inhibiting PLC. The induction of F-actin breakdown or AR by PBP10 is not affected by inhibiting PLC, indicating the specificity of PLC inhibition to PIP_{2} hydrolysis.

It is well known that there is high increase in intracellular Ca^{2+} concentrations as a result of the interaction between capacitated spermatozoa.
sperm and the egg zona pellucida.29 This increase in calcium is essential for the occurrence of the AR which is known to be mediated by PLC activity.30 Interestingly, activation of gelsolin by PBP10 in capacitated sperm, which induces the AR, occurs under conditions in which intracellular Ca\(^{2+}\)-concentration is relatively low, further indicating that the increase in intracellular Ca\(^{2+}\)is essential for the activation of PLC to hydrolyse PIP\(_2\), leading to the release of bound gelsolin, thereby activating this molecule and stimulating the depolymerization of F-actin, an essential step for the AR to occur.

**GELSON ACTIVITY IS INHIBITED DURING CAPACITATION**

Since F-actin levels are increased during capacitation, it is likely that gelsolin is inactive during this period of time. Gelsolin can be inhibited by its Src-dependent phosphorylation on tyr-438 and/or its binding to PIP\(_2\), two processes that occur during sperm capacitation. In human sperm, Src is involved in regulating capacitation, Ca fluxes, protein tyrosine phosphorylation and the AR.31-33 Previous studies suggested that Src is not directly involved in protein tyrosine phosphorylation but rather inhibits protein phosphatase resulting in an increase in tyrosine phosphorylation of proteins.34 It was shown elsewhere that capacitation is regulated by two parallel pathways, activation of PKA activates Src leading to inactivation of Ser/Thr phosphatase.35,36 (Figure 3). We have recently shown that activation of Src by PKA, inhibits the Ser/Thr phosphatase PP1 resulting in CaMKII activation leading to activation of Pyk2 which phosphorylates PI3K on tyrosine-845.3 It was found in sperm tail and head and is localized to the membrane fraction,37 similar to gelsolin localization and consistent with our assumption about its inactivating function. These assumptions were verified when Src-dependent phosphorylation of gelsolin on tyr-438 during sperm capacitation was found.38 In addition, we found that activation of PKA/Src caused F-actin formation, which was inhibited by inhibiting Src activity,32 suggesting that activation of Src causes gelsolin inhibition and an increase in F-actin. Also, PBP10-induced F-actin depolymerization is inhibited by activating Src or by inhibition of tyrosine-phosphatase suggesting that although gelsolin is released from its binding to PIP\(_2\), it is still highly phosphorylated and inactive. The levels of tyr-p-gelsolin are enhanced by elevating the cellular levels of PIP\(_2\), and vice versa,35 suggesting that binding of gelsolin to PIP\(_2\) increases its phosphorylation/inhibition. These findings are further supported by showing a decrease in tyr-p-gelsolin by activation of PLC to hydrolyse PIP\(_2\), or by releasing gelsolin from PIP\(_2\) using PBP10. These observations also suggest that free tyr-p-gelsolin is more sensitive to tyr-phosphatase activity compared to the PIP\(_2\)-bound p-gelsolin. Also, activation of sperm EGFR caused PLC-dependent dephosphorylation of p-gelsolin, after 1 h of incubation under capacitation conditions.34 These results confirm our hypothesis that gelsolin is inhibited during capacitation due to its binding to PIP\(_2\), and tyr-phosphorylation by Src.

**In conclusion, our data suggest the following model (Figures 4 and 5): the**

![Figure 4](Image 444x20 to 462x37)

**Table 1: Effect of various factors on actin modulation during sperm capacitation**

|                | Time (min) | Relative fluorescence (percentage of control) |
|----------------|------------|-----------------------------------------------|
| Control        | 0          | 100±14.80                                     |
| Phosphatidic acid | 3          | 188±15.12                                     |
| Control        | 30         | 98±14.75                                      |
| Spermin        | 30         | 212±11.74                                     |
| Wortmannin     | 30         | 95±22.42                                      |
| Control        | 180        | 185±16.20                                     |
| PBP10          | 180        | 111±11.77                                     |
| Wortmannin     | 180        | 112±6.839                                     |

![Figure 5](Image 256x136 to 511x278)

In conclusion, our data suggest the following model (Figures 4 and 5): the
relatively small increase in \([\text{Ca}^{2+}]\), during capacitation leads to conformational changes in gelsolin revealing the F-actin binding site. This change and the increase in F-actin and PIP_2 in the sperm head, result in gelsolin being translocated to this region of the cell. Nevertheless, the elevation of PIP_2 levels and PKA/Src activation, maintain gelsolin in a phosphorylated/inactivated state and actin polymerization occurs. The increase in F-actin in the tail leads to the development of hyper-activated motility as part of the capacitation process. Immediately prior to the AR (Figure 5), the egg zona-pellucida activates GPCR^11^ and/or the EGFR^14^ leading to an increase in [Ca^2+], and activation of PLC to hydrolyse PIP_2, resulting in the release of PIP_2-bound gelsolin which undergoes dephosphorylation/activation by tyrosine-phosphatase, leading to F-actin dispersion in the head and the occurrence of the AR.

REFERENCES

1. Visconti PE. Understanding the molecular basis of sperm capacitation through kinase design. Proc Natl Acad Sci U S A 2009; 106: 667–8.
2. Buffone MG, Wertheimer EV, Visconti PE, Krapt D. Central role of soluble adenyl cyclase and cAMP in sperm physiology. Biochim Biophys Acta 2014; 1842 (12 Pt B): 2610–20.
3. Rotfeld H, Hiilam P, Ickowicz D, Brittart H, Pka and CaMKII mediate PI3K activation in bovine sperm by inhibition of the PKC/PPI cascade. Reproduction 2014; 147: 347–56.
4. Brener E, Rubinstein S, Cohen G, Shternall K, Rivlin J, et al. Remodeling of the actin cytoskeleton during mammalian sperm capacitation and acrosome reaction. Biol Reprod 2003; 68: 387–45.
5. Brittart H, Cohen G, Rubinstein S. Role of actin cytoskeleton in mammalian sperm capacitation and the acrosome reaction. Reproduction 2005; 129: 263–8.
6. Spungin B, Margalit I, Brittart H. Sperm exocytosis reconstructed in a cell-free system: evidence for the involvement of phospholipase C and actin filaments in membrane fusion. J Cell Sci 1995; 108 (Pt 6): 2525–35.
7. Arndt CA, Kazam IG, Visconti PE, Kopf GS, Villar M, et al. Control of the low voltage-activated calcium channel of mouse sperm by egg ZP3 and by membrane hyperpolarization during capacitation. Proc Natl Acad Sci U S A 1999; 96: 6757–62.
8. Itach SB, Finkelstein M, Etkowitz N, Brittart H. Hyper-activated motility in sperm capacitation is mediated by phospholipase D-dependent actin polymerization. Dev Biol 2012; 362: 154–61.
9. Demott RP, Suarez SS. Hyperactivated sperm progress in the mouse oviduct. Biol Reprod 1992; 46: 779–85.
10. Katz DF, Vanaginich M. Movement characteristics of hamster spermatozoa within the oviduct. Biol Reprod 1980; 22: 759–64.
11. Ho HC, Suarez SS. Hyperactivation of mammalian spermatozoa: function and regulation. Reproduction 2001; 122: 513–26.
12. Brown HA, Gutowsk S, Moomaw CR, Slahter C, Sternewase PC, ADP-riboisyltransferase, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. Cell 1993; 75: 1137–44.
13. Liscovich M, Chalifa V, Pertle P, Chen CS, Cantley LC. Novel function of phosphatidylinositol 4,5-bisphosphate as a cofactor for brain membrane phospholipase D. J Biol Chem 1994; 269: 21403–6.
14. Pertle P, Liscovich M, Chalifa V, Cantley LC. Phosphatidylinositol 4,5-bisphosphate synthesis is required for activation of phospholipase D in U937 cells. J Biol Chem 1995; 270: 5130–5.
15. Schmidt M, Rümenapp U, Nehls C, Ott S, Keller J, et al. Restoration of Clostridium difficile toxin-B–inhibited phospholipase D by phosphatidylinositol 4,5-bisphosphate. Eur J Biochem 1996; 240: 707–12.
16. Hodgkin MN, Masson MR, Power D, Saqib KM, Ponting CP, et al. Phospholipase D regulation and localisation is dependent upon a phosphatidylinositol 4,5-bisphosphate-specific PH domain. Curr Biol 2000; 10: 43–6.
17. Dí Paolo G, De Camilli P. Phosphoinositides in cell membrane and membrane dynamics. Nature 2006; 443: 651–7.
18. Yin HL, Janney PA. Phosphoinositide regulation of the acrosine cytoskeleton. Annu Rev Physiol 2003; 65: 761–89.
19. Etkowitz N, Rubinstein S, Daniel L, Brittart H. Role of PI3-kinase and PI4-kinase in actin polymerization during bovine sperm capacitation. Biol Reprod 2007; 77: 263–73.
20. Jungnickel MK, Sutton KA, Wang Y, Florman HM. Phosphoinositide-dependent pathways in mouse sperm are regulated by egg ZP3 and drive the acrosome reaction. Dev Biol 2007; 304: 116–26.
21. JanneyPA, Lida K, Yin HL, Stossel TP. Phosphoinositide micelles and phosphoinositide-containing vesicles dissociate endogenous gelsolin-actin complexes and promote actin assembly from the fast-growing end of actin filaments blocked by gelsolin. J Biol Chem 1987; 262: 12228–36.
22. Finkelstein M, Etkowitz N, Brittart H. Role and regulation of sperm gelsolin prior to fertilization. J Biol Chem 2010; 285: 39702–9.
23. Finkelstein M, Megnoli B, Ickowicz D, Brittart H. Regulation of sperm motility by PI(3,4) and actin polymerization. Dev Biol 2013; 381: 62–72.
24. Gremm D, Wegner A. Gelsolin as a calcium-regulated actin filament-capping protein. Eur J Biochem 2000; 267: 4339–45.
25. Yin HL. Gelsolin: calcium- and polyphosphoinositide-regulated actin-modulating protein. Bioessays 1987; 7: 176–9.
26. De Corte V, Demol H, Goethals M, Van Damme J, Gettemans J, et al. Identification of Tyr438 as the major in vitro e-Src phosphorylation site in human gelsolin: a mass spectrometry approach. Protein Sci 1999; 8: 234–41.
27. Kinoshita J, Newman J, Lucinon B, Selden LA, Gershman LC, et al. Ca2 regulation of gelsolin activity: binding and seversing of F-actin. Biophys J 1998, 75: 3101–9.
28. Guttman JA, Janney P, Vogl AW. Gelsolin – evidence for a role in turnover of junction-related actin filaments in Sertoli cells. J Cell Sci 2002; 115 (Pt 3): 499–505.
29. Florman HM. Sequential focal and global elevations of sperm intracellular Ca2 are initiated by the zona pellucida during acrosomal exocytosis. Dev Biol 1994; 165: 152–64.
30. Etkowitz N, Tiros Y, Chazan R, Jaldety Y, Daniel L, et al. Bovine sperm acrosome reaction induced by G-protein-coupled receptor agonists is mediated by epidermal growth factor receptor transactivation. Dev Biol 2009; 334: 447–57.
31. Varano G, Lombardi A, Cantini G, Forti G, Baldi E, et al. Src activation triggers capacitation and acrosome reaction but not matility in human spermatozoa. Hum Reprod 2008; 23: 2652–62.
32. Baker MA, Hetherington L, Atken RJ. Identification of SRC as a key PKA-stimulated tyrosine kinase involved in the capacitation-associated hyperactivation of murine spermatozoa. J Cell Sci 2006; 119 (Pt 15): 3182–92.
33. Mitchell LA, Nixon B, Baker MA, Hetherington L, Atken RJ. Investigation of the role of SRC in capacitation-associated tyrosine phosphorylation of human spermatozoa. Mol Hum Reprod 2008; 14: 235–43.
34. Krapf D, Arcelay E, Wertheimer EV, Sanjay A, Pilder SH, et al. Inhibition of Ser/Thr phosphatases induces capacitation-associated signaling in the presence of Src kinase inhibitors. J Biol Chem 2010; 285: 7797–85.
35. Lawson C, Goupil S, Leclerc P. Increased activity of the human sperm tyrosine kinase SRC by the cAMP-dependent pathway in the presence of calcium. Biol Reprod 2008; 79: 657–66.
36. Florman HM, Tombee RM, First NL, Babcock DF. An adhesion-associated agonist from the zona pellucida activates G protein-promoted elevations of internal Ca2 and pH that mediate mammalian sperm acrosomal exocytosis. Dev Biol 1989; 135: 133–46.
37. Jaldety Y, Glick Y, Orr-Urtreger A, Ickowicz D, Gerber D, et al. Sperm epidermal growth factor receptor (EGFR) mediates a7 acetylcholine receptor (AChR) activation to promote fertilization. J Biol Chem 2012; 287: 23238–40.