Zygote 8 (Supplement), pp. S28–S30. © 2000 Cambridge University Press Printed in the United Kingdom

suREJ proteins: new signalling molecules in sea urchin spermatozoa

Kathryn J. Mengerink, Gary W. Moy and Victor D. Vacquier
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0202, USA

In *Strongylocentrotus purpuratus*, the fucose sulphate polymer (FSP) of egg jelly induces the sperm acrosome reaction (AR; Vacquier & Moy, 1997). Protease treatment of sperm renders the cells insensitive to FSP, indicating that sperm membrane receptors mediate the signal transduction events underlying the AR. Monoclonal antibodies to a 210 kDa membrane glycoprotein induce Ca^{2+} influx into sperm and trigger the AR (Trimmer *et al.*, 1986; Moy *et al.*, 1996). Purified 210 kDa protein binds species-specifically to egg jelly and blocks AR induction by antibody (Podell & Vacquier, 1985; Moy *et al.*, 1996). FSP binds to the 210 kDa protein attached to Sepharose (Vacquier & Moy, 1997). Monoclonal antibodies localise the 210 kDa protein on the plasma membrane over the acrosome and also on the sperm flagellum. The 210 kDa protein has the attributes of a sperm receptor for egg jelly and is henceforth named suREJ1 (Moy *et al.*, 1996). We describe here the three REJ proteins found thus far in *S. purpuratus* sperm.

suREJ1

suREJ1 is 1450 amino acids and has an expected protein mass of approximately 160 kDa. Western blots of HF-deglycosylated sperm membrane proteins, probed with an IgG to a suREJ1-specific peptide, show a single reaction at approximately 160 kDa (Moy *et al.*, 1996). Glycosylation represents approximately 50% of the protein's mass (Podell & Vacquier, 1985). Beginning at the amino-terminus, suREJ1 consists of one EGF module of 40 residues, followed by two carbohydrate recognition domain (CRD) modules (120 residues each) of the C-type lectin variety. The two CRDs are divergent, being only 50% identical in amino acid sequence (Moy *et al.*, 1996). suREJ1 has one putative transmembrane segment (TMS) at its extreme carboxyl-terminus, with only 15 residues being putatively cytoplasmic. Treatment of sea urchin sperm with IgG to suREJ1 causes the translocation of the protein in the plane of the membrane, suggesting it is not strongly attached to cytoplasmic proteins (Trimmer & Vacquier, 1988). Sixty to seventy per cent of suREJ1 antigenicity becomes 200 000 *g* soluble when sperm are treated for 5 h with pH 9.1 seawater. This also shows a lack of tenacious association of suREJ1 with the sperm plasma membrane. That monoclonal antibodies to suREJ1 cause Ca^{2+} influx into sperm and induce the AR implicates this protein as an important signal transducer in sperm (Moy *et al.*, 1996).

suREJ2

One suREJ clone was found that was 82% identical to suREJ1 for 400 amino acids. The full-length sequence of this second REJ (suREJ2) is 1472 amino acids. It is quite different from suREJ1 in its amino-terminal modules. suREJ2 does not have an EGF module, but does have one CRD. Downstream of the CRD there are eight repeats of 12 residues, which are considerably hydrophobic. Next, comes an insertion of approximately 100 residues not found in suREJ1. The extreme carboxyl-terminus of suREJ2 is similar to that of suREJ1 with a single TMS and 15 residues putatively cytoplasmic. Peptide antibodies specific to suREJ2 react with one protein of approximately 150 kDa on western blots of both untreated and HF-deglycosylated sperm membrane proteins, suggesting that suREJ2 is not extensively glycosylated. Also, suREJ2 does not bind WGA lectin, whereas suREJ1 does. A peptide-specific antibody to suREJ2 localises the protein to the mitochondrial midpiece and flagellum, but not to the acrosomal region of the sea urchin sperm.

suREJ3

While sequencing suREJ2 clones, one clone was found which was neither suREJ1 nor suREJ2. Completion of this sequence shows suREJ3 is 2675 residues. From amino- to carboxyl-terminus, it has one SUEL domain (sea urchin egg lectin domain, ~105 residues; Ozeki *et al.*, 1995). Contiguous with the SUEL domain is one CRD which is 40–46% identical to the suREJ1 and

https://doi.org/10.1017/S0967199400130138 Published online by Cambridge University Press

suREJ2 CRDs. Downstream of the CRD is the 100 residue insertion similar to the one found in suREJ2. A hydropathy plot of the carboxyl-terminal half of suREJ3 indicates 11 putative TMS (compared with one TMS of the other two REJ proteins). Between TMS1 and TMS2 is a domain homologous to the amino-terminal domain of lipoxygenases. An antibody to a 17mer peptide unique to the suREJ3 CRD localises the protein to the sperm head plasma membrane covering the acrosome.

Relationship of suREJ proteins to PKD proteins and latrophilins

The single TMS of suREJ1 and suREJ2 aligns with the first TMS of suREJ3. Upstream from this TMS, for approximately 1000 residues, these three proteins share homology with human polycystin (huPKD1), the protein mutated in 85% of autosomal dominant polycystic kidney disease. For example, in this region suREJ1 and huPKD1 are 20% identical and 40% similar. A random jumble analysis shows the homology to be statistically significant (Moy *et al.*, 1996). huPKD1 is 4302 residues and contains a lectin-like module, leucine-rich region, cysteine-rich region, LDL receptor module and 16 repeats of approximately 100 residues specific to PKD1 called 'PKD1 repeats' (International Polycystic Kidney Disease Consortium, 1995). The 100 residue insertions found in suREJ2 and suREJ3, but not suREJ1, are homologous to the 'PKD repeats'. In suREJ3, downstream of the first TMS and for the remainder of the protein, there is significant homology to the 11 TMS of huPKD1 and other PKD family members such as PKDREJ, PKD2 and PKD-L. About 2500 residues of huPKD1 are predicted to be extracellular. huPKD1 has the characteristics of a membrane-associated carbohydrate binding protein (International Polycystic Kidney Disease Consortium, 1995; Ward *et al.*, 1996; Ibraghimov-Beskrovnaya *et al.*, 1997). huPKD1 is expressed in many human tissues (Ward *et al.*, 1996); its function remains unknown.

PKDREJ (Hughes *et al.*, 1999) is the mammalian homologue most closely related to suREJ3. This protein is slightly shorter than suREJ3. It does not have a SUEL domain, a CRD, nor any 'PKD repeats' characteristic of huPKD1, suREJ2 and suREJ3. However, PKDREJ does possess approximately 1000 extracellular residues and the 11 putative TMS characteristic of PKD proteins and suREJ3. In mouse, PKDREJ is expressed only in the testis and only during the time of sperm differentiation. These observations implicate PKDREJ as a signalling protein of mammalian sperm (Hughes *et al.*, 1999). Hydropathy plots of huPKD1, PKDREJ and suREJ3 show that the 11 TMS of these proteins are homologous. The carboxyl-terminus of suREJ3, huPKD1 and PKDREJ, comprising TMS 6-11, is homologous to huPKD2, voltage-dependent Ca^{2+} channels and TRP (transient receptor potential channels; Montell, 1997). TRPC1, a mammalian TRP channel, has been shown to associate with huPKD2 (Tsiokas *et al.*, 1998). Also, a PKD2 homologue, PKD-L, has been shown to form Ca^{2+}-activated cation channels when expressed in *Xenopus* oocytes (Chen *et al.*, 1999).

Latrophilins are G-protein coupled, 7 TMS receptors. Latrophilin-1 binds α-latrotoxin (black widow spider venom) inducing the exocytosis of synaptosomes in mammals (Sugita *et al.*, 1998; Krasnoperov *et al.*, 1997). suREJ3 shares several features with latrophilins. For example, the SUEL domain of approximately 105 residues, originally described in sea urchin eggs (Ozeki *et al.*, 1995), is the most conserved feature of latrophilins. Latrophilins are post-translationally cleaved within a conserved sequence domain in the extracellular part of the protein just before their first TMS. The three sea urchin REJ proteins possess this conserved latrophilin cleavage site as do other members of the G-protein coupled receptor family. The two cleavage products of latrophilins tightly associate on the outside surface of the cell (Sugita *et al.*, 1998; Krasnoperov *et al.*, 1997). Preliminary western blots, probed with two antibodies reacting on different sides of the suREJ3 putative latrophilin cleavage site, identify single reacting bands of different sizes, providing evidence that suREJ3 is in fact post-translationally cleaved in mature sperm.

Following the first TMS of suREJ3 are 125 cytoplasmic residues sharing homology with the amino-terminal end of lipoxygenases. Lipoxygenases comprise a large family of proteins that peroxidise lipids (Kuhn & Thiele, 1999). huPKD1 and PKDREJ also share this homology. The extracellular latrophilin cleavage site, followed by putative TMS-1, which is in turn followed by the intracellular lipoxygenase domain, indicates that the location of putative TMS-1 is correctly assigned in suREJ3 and PKD family members.

We predict that suREJ3 and mammalian PKDREJ, will prove to be key players in the induction of the sperm AR. The homology of TMS 6-11 to known channel proteins suggests that they are members of this new family of channel-forming proteins. Their close relationship with each other, and with the latrophilins, suggests that the transmembrane signalling events underlying the sperm AR are conserved among the deuterostomes.

Supported by NIH Grant HD12986.

References

Chen, X.Z., *et al.* (1999). *Nature* **401**, 383–6.
Hughes, J., *et al.* (1999). *Hum. Mol. Genet.* **8**, 543–9.
Ibraghimov-Beskrovnaya, O., *et al.* (1997). *Proc. Natl. Acad.*

Sci. USA **94**, 6397–402.
International Polycystic Kidney Disease Consortium. (1995). *Cell* **81**, 289–98.
Krasnoperov, V.G., *et al.* (1997). *Neuron* **18**, 925–37.
Kuhn, H. & Thiele, B.J. (1999). *FEBS Lett.* **449**, 7–11.
Montell, C. (1997). *Mol. Pharmacol.* **52**, 755–63.
Moy, G.W., *et al.* (1996). *J. Cell Biol.* **133**, 809–17.
Ozeki, Y., *et al.* (1995). *Exp. Cell Res.* **216**, 318–24.
Podell, S.B. & Vacquier, V.D. (1985). *J. Biol. Chem.* **260**, 2715–18.
Sugita, S., *et al.* (1998). *J. Biol. Chem.* **273**, 32712–24.
Trimmer, J.S. & Vacquier, V.D. (1988). *Exp. Cell Res.* **175**, 37–51.
Trimmer, J.S., Schackmann, R.W & Vacquier, V.D. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 9055–9.
Tsiokas, L., *et al.* (1998). *Proc. Natl. Acad. Sci. USA* **96**, 3934–9.
Vacquier, V.D. & Moy, G.W. (1997). *Dev. Biol.* **192**, 125–35.
Ward, C.J., *et al.* (1996). *Proc. Natl. Acad. Sci. USA* **93**, 1524–8.

https://doi.org/10.1017/S0967199400130138 Published online by Cambridge University Press