The Electrical Polyspermy Block

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At fertilization, one sperm and one egg combine to form an embryo; if two or more sperm fertilize an egg, the embryo fails to develop. The problem of polyspermy prevention has been solved in diverse ways in different organisms.[1] One common mechanism is the electrical block to polyspermy.

Since the cellular events of fertilization were first observed over 100 years ago, it was clear that one component of the block to polyspermy in organisms such as sea urchins, starfish, and frogs is the elevation of a protective envelope around the egg, the fertilization envelope. However, there was controversy as to whether this relatively slow process, which takes about one minute, was fast enough to block polyspermy completely. There was speculation that since fast events in nerve and muscle were electrically mediated, an electrical mechanism might provide a fast block to polyspermy, before the fertilization envelope is fully elevated. The tools to make electrical measurements in eggs were not available until the 1950s, when an electrical change at fertilization—now called the fertilization potential —was first measured, in the egg of a starfish.[2] At this time, the fertilization potential was described, but its function was not explored further.

The discovery of the electrical polyspermy block is a story of serendipity; it began with a project having nothing to do with polyspermy. I was a graduate student in the laboratory of Dr. Susumu Hagiwara at UCLA, and at that time, there was great interest in the idea that an influx of calcium might activate the egg at fertilization. With this in mind, Dr. Hagiwara suggested that I try to block egg activation at fertilization by clamping the voltage of the sea urchin egg membrane at a very positive potential, so that positively charged calcium ions could not enter the egg. This experiment was based on an earlier experiment[3] which showed that holding the voltage across the nerve terminal membrane at about +200 mV blocked calcium entry and therefore blocked synaptic transmission. Could fertilization also be blocked?

Using a fine microelectrode inserted through the egg membrane to pass current, I held the voltage of the egg membrane at about +200 mV. When sperm were added, the neighboring eggs fertilized, but the voltage-clamped egg did not! Was this because calcium entry was blocked? To test this, I held the voltage of the egg membrane at successively less positive potentials, to determine the threshold required to block fertilization. In the nerve terminal, potentials greater than about +100 mV are required before inhibitory effects on calcium entry are seen. However, in the sea urchin egg, potentials as small as +5 mV inhibited fertilization. The work with nerves had shown that calcium entry would not be significantly blocked at +5 mV, so the result that I was seeing was clearly unrelated to what I had set out to find originally.

At this point it occurred to me that my findings might be related to the old idea of an electrically mediated polyspermy block. +5 mV was approximately the voltage attained during the fertilization potential. Could it be that by holding the egg's membrane potential at +5 mV, I was mimicking the fertilization potential and that the natural function of the fertilization potential was to block polyspermy?

If this was so, I realized that it should be possible to induce polyspermy by suppressing the positive potential shift. After observing the rise of the fertilization potential, I applied current to bring the egg's membrane potential back to −30 mV. When such eggs were observed 2 hours later, they were seen to have cleaved into 3 cells, a clear indicator of polyspermy.

These findings[4] opened up many new questions. Did this mechanism operate in other organisms as well? How was the positive potential suppressing fertilization? In the past 15 years we have partially answered these questions.

The electrical polyspermy block functions in many but not all species; it even operates in at least one plant species, the sea weed *Fucus*.[5] In most cases, a positive shift in potential accounts for the block, but in crabs, the block occurs as a result of a negative shift in potential.[6]

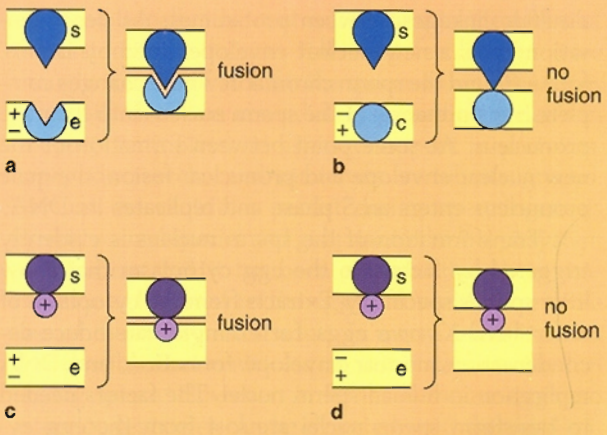

Figure A Two models of how the egg's membrane potential might regulate sperm-egg fusion. Parallel lines represent the sperm (s) and egg (e) plasma membranes; the solid figures represent membrane molecules important in fusion. **(a, b)** Potential-controlled conformational change in an egg membrane molecule. When the egg's membrane potential is negative **(a)** the successful interaction of egg and sperm molecules leads to membrane fusion. Positive potential **(b)** prevents fusion. **(c, d)** Potential-controlled insertion of a sperm membrane molecule into the egg plasma membrane. When the egg's membrane potential is negative **(c)** the positively charged portion of the sperm molecule inserts fully and fusion follows. Positive potential **(d)** inhibits full insertion and prevents fusion. Redrawn from *Ann. Rev. Physiol.* 48:191 (1986).

The evidence so far indicates that electrical polyspermy blocks do not occur in mammals; sperm access to mammalian eggs may occur slowly enough that a fast electrical mechanism for polyspermy prevention is not needed.

As for the mechanism by which membrane potential regulates the fusion of the sperm with the egg, we have made two important findings.[7] First, the block to fertilization is due to the voltage change itself and not to the accompanying ion movements. Second, and surprisingly, the "voltage sensor" appears to be in sperm rather than the egg. In principle, the voltage sensitivity of fertilization could result from a receptor molecule in the egg membrane that undergoes a conformational change from receptive to nonreceptive in response to a change in transmembrane electrical potential (Fig. A*a, b*). Ion channels in cell membranes open and close by this means. However, this does not seem to be the case for fertilization. Instead, it appears that the voltage-sensitivity of fertilization results from a voltage sensor in the sperm, perhaps a positively charged region of a sperm membrane protein that must insert in the egg membrane to initiate sperm-egg fusion (Fig. A*c, d*). The idea is that membrane insertion of such a positively charged peptide would be favored if the potential on the inside of the egg membrane is negative and opposed if it is positive.

Evidence for this model came from a series of cross-fertilization experiments between different animal species that showed differing degrees of voltage-dependence in fertilization. In some species, fertilization occurs with the same probability regardless of the voltage across the egg membrane; an example is the Japanese salamander *Cynops*. *Cynops* eggs can, in the laboratory, be fertilized with sperm from another Japanese salamander *Hynobius*. Unlike *Cynops*, *Hynobius* fertilization is blocked by a positive egg membrane potential.

During a visit to Yamaguchi University in Japan, I collaborated with Dr. Yasuhiro Iwao to find out whether the cross-fertilization of *Cynops* eggs by *Hynobius* sperm was voltage-dependent.[8] We voltage-clamped *Cynops* egg at positive potentials and then added *Hynobius* sperm; we found that positive but not negative potentials inhibited *Hynobius* sperm entry. Since fertilization of *Cynops* eggs by *Cynops* sperm is voltage-independent, we concluded that the voltage-sensitivity could only have come from the *Hynobius sperm*. Together with earlier work showing that fertilization of a voltage-sensitive egg species by a voltage-insensitive sperm species is voltage *insensitive*, this result demonstrated that the voltage sensor must be in the sperm.

The challenge now is to identify this voltage-sensitive molecule. Perhaps it will be a fusion protein like the fusion proteins that mediate virus-host cell fusion.[9] Viral fusion proteins have a hydrophobic region that inserts in the host cell membrane. If this hydrophobic region included some positively charged amino acids such as arginine or lysine, its insertion into a membrane would be voltage-dependent. The presence or absence of these charged amino acids could determine whether fertilization in a particular species was or was not voltage-dependent.

References

[1] Jaffe, L. A., and Gould, M. In *Biology of Fertilization*, C. B. Metz and A. Monroy, eds. Orlando: Academic Press, p. 223 (1985).

[2] Tyler, A.; Monroy, A.; Kao, C. Y.; and Grundfest, H. *Biol. Bull.* 111:153 (1956).

[3] Katz, B., and Miledi, R. *J. Physiol.* 192:407 (1967).

[4] Jaffe, L. A. *Nature* 261:68 (1976).

[5] Brawley, S. H. *Devel. Biol.* 144:94 (1991).

[6] Goudeau, H., and Goudeau, M. *Devel. Biol.* 133:348 (1989).

[7] Jaffe, L. A., and Cross, N. L. *Ann. Rev. Physiol.* 48:191 (1986).

[8] Iwao, Y., and Jaffe, L. A. *Devel. Biol.* 134:446 (1989).

[9] White, J. M. *Ann. Rev. Physiol.* 52:675 (1990).