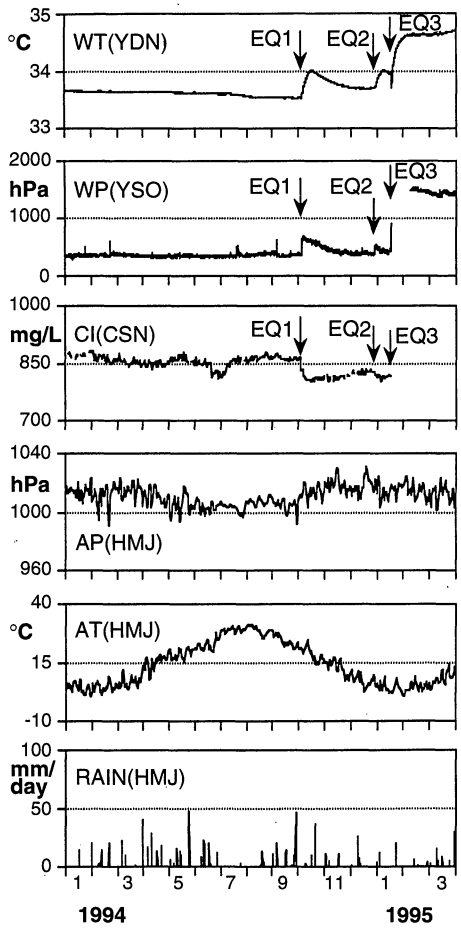

Fig. 2. Temporal variations of water temperature recorded at YDN, water pressure at YSO, chloride-ion content at CSN, and atmospheric pressure, temperature, and rainfall at HMJ. Earthquakes 1, 2, and 3 occurred on 4 October 1994, 28 December 1994, and 17 January 1995 (Kobe), respectively.

better opportunity to study statistically their correlation. Only when a sufficient number of robust cases have been observed in Kobe and elsewhere may we conclude whether a geochemical or geophysical method is useful for prediction. Also, because of the complexity of Earth's crust, it is unlikely that any single method will be useful everywhere. Thus, a multidisciplinary approach for prediction is needed, along with other measures for earthquake-hazard mitigation.

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The Complexities of Conception

R. John Aitken

At insemination, some 200 million spermatozoa are released into the female reproductive tract. Their task: to find just one other cell—the egg. After their journey "upstream" to the Fallopian tubes, the first contact between male and female gametes appears to be a disconcertingly random affair. But, once made, contact is maintained by an exquisitely tuned cell-specific recognition process that results in the tenacious binding of the spermatozoon to an extracellular shell surrounding the oocyte, the zona pellucida (see figure). After the spermatozoon has become tightly bound to the zona pellucida, it is induced to undergo the acrosome reaction, a secretory event that facilitates the spermatozoon's passage through the zona pellucida and its subsequent fusion with the vitelline membrane of the oocyte (see figure). In this week's issue of *Science*, two reports consider the molecular basis of this process. However, the conclusions that they reach are very different.

The molecule on the surface of the ovum responsible for binding and activating spermatozoa is a major glycoprotein constituent of the zona pellucida, ZP3 (*1*). However the nature of the complementary receptors on the sperm surface that bind ZP3, and the biochemistry of the subsequent signal tranduction events, are still open to question. One class of molecule that could account for both ZP3 recognition and signal transduction is a receptor tyrosine kinase (*2*). Exposure of both mouse and human spermatozoa to ZP3 results in the rapid autophosphorylation of tyrosine residues on a putative zona receptor kinase (ZRK) of 95 kilodaltons. In one of the reports in this issue, Burks and coworkers (*3*) used a monoclonal antibody directed against this molecule to screen a human testicular complementary DNA (cDNA) library and isolate a full-length clone predicting a 600–amino acid receptor that contains a unique cysteine-rich extracellular domain. Peptides from the extracellular domain of this molecule suppress sperm-zona binding, suggesting a role for ZRK in gamete recognition as well as signal transduction.

Tyrosine phosphorylation is an important component of the signal transduction cascade used by mammalian spermatozoa with agonists such as progesterone and PAF (*4, 5*), as well as ZP3. However, a central role for tyrosine kinases in sperm-egg recognition is more difficult to understand. This difficulty arises because tyrosine kinase receptors are generally targeted by proteinaceous ligands, and yet there is abundant evidence to suggest that it is the oligosaccharide side chains of ZP3 that mediate the first contact between the sperm plasma membrane and the zona surface. In this context, mouse spermatozoa appear to possess a particular affinity for terminal galactose residues on one class of O-linked oligosaccharides on ZP3 (*6*). Removal or modification of this sugar residue results in a loss of sperm-binding activity. The use of cross-linking and affinity chromatography strategies has demonstrated that this class of oligosaccharide binds to a single lectin-like molecule, localized on the sperm head, with a molecular mass of 56 kilodaltons (sp56) (*7*). In the second report in this issue, Bookbinder *et al.* describe the cloning and sequencing of a full-length cDNA for this molecule (*8*). The cDNA encodes a 547–amino acid peripheral membrane protein with no transmembrane domain and no obvious sequence homology to other galactose-binding proteins such as the rat liver asialoglycoprotein receptor (RHL 2/3). However, this protein is homologous to members of a superfamily of protein receptors of which the most closely related appears to be the α subunit of the complement 4B binding protein.

Evidence that sp56 is a ZP3 receptor in the mouse is convincing, although it is not the only candidate for this role. A sperm-associated galactosyltransferase has also been nominated; this enzyme targets terminal *N*-acetylglucosamine residues on the zona pellucida oligosaccharide side chains, rather than the galactose residues bound by sp56 (*9*). Moreover, aggregation of this receptor induces the acrosome reaction through the activation of a $G_i\alpha$-containing heterotrimeric complex (*10*), providing an alternative signal transduction mechanism to the tyrosine kinase pathway suggested by Burks *et al.* (*3*). Lectins have also been implicated in the binding of human spermatozoa to ZP3, although in this case the sugar involved is neither galactose nor *N*-acetylglucosamine, but mannose (*11*). Human spermatozoa are devoid of both sp56 and significant galactosyltransferase activity but do possess a D-mannosidase activity, which

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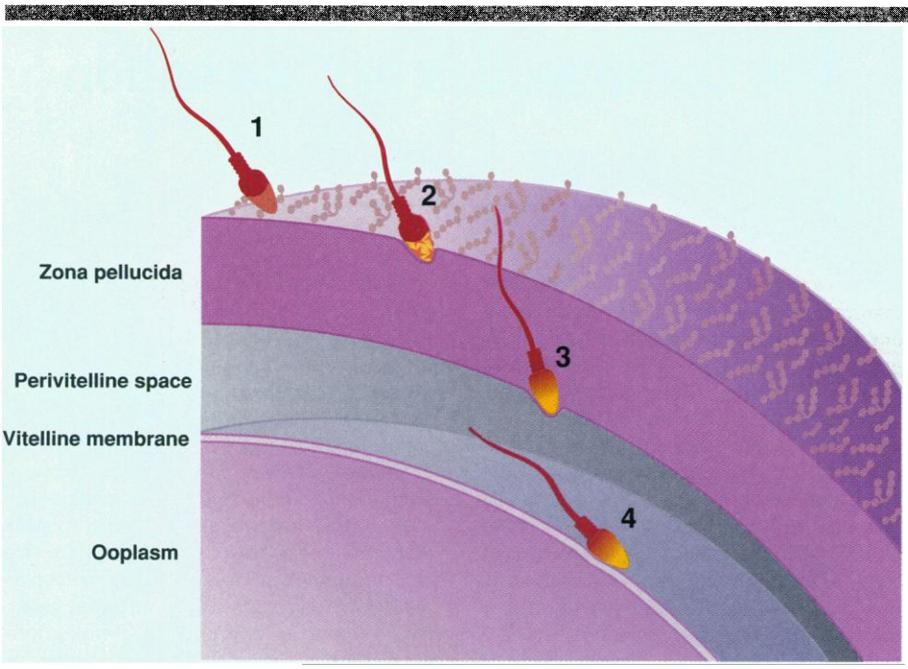

Model of sperm-egg fusion. 1, Primary adhesion between the vigorously motile spermatozoon and the bulky carbohydrate groups projecting from the zona surface may be mediated by one or more sperm surface lectins such as sp56. Because the latter is localized over the anterior aspect of the sperm head, it also orients the spermatozoon for zona penetration. 2, Receptor tyrosine kinase (ZRK) may then be involved in establishing the tight binding of the spermatozoon to the zona surface and initiating a signal transduction cascade that precipitates the acrosome reaction. 3, The acrosome-reacted cell penetrates through the zona pellucida into the perivitelline space. 4, Spermoocyte fusion is initiated by the plasma membrane overlying the equatorial segment of the sperm head.

may be involved in binding ZP3 (*12*). An alternative candidate for the human sperm mannose receptor is an unusual C-type lectin linked through a membrane-spanning α-helical domain to a cytoplasmic tail bearing actin-binding motifs (*13*). Yet another lectin, Sp17, is thought to mediate sperm-zona recognition in rabbit and human spermatozoa, but at a much later stage in fertilization, after the acrosome reaction (*14*).

This issue of *Science* therefore contains important sequence data on two disparate molecules, each claimed to be mediators of sperm-egg interaction during the early stages of fertilization. Of course, these data are not necessarily conflicting. They may simply indicate that the binding and activation of spermatozoa at the zona surface is a multiphasic event that requires a number of different receptors interacting with different parts of the ZP3 molecule in sequence. An analogous process may be the endothelial cell–leukocyte adhesion cascade of inflammatory reactions. In the initial stages of this process, adhesive contact between neutrophils and the endothelial surface is mediated by a family of lectin-like molecules (selectins). This initial loose adhesion is then followed by tenacious binding and the initiation of a signal transduction cascade through the engagement and activation of an additional set of adhesion molecules, including β-integrins and ICAMs (intracellular adhesion molecules).

If this cascade were parallel to the sperm-zona interaction, the initial adhesion phase would be mediated by lectin-like components, such as sp56 or galactosyl transferase, interacting with the bulky ZP3 oligosaccharide side chains protruding from the surface of the zona pellucida. The purpose of this initial lectin-mediated adhesion would be to trap the vigorously motile spermatozoon at the zona surface and orient it in such a way that penetration will occur. This event might then be followed by additional interactions involving the binding of ZRK to the polypeptide core of ZP3 that would enhance the tenacity of the binding and, in concert with alternative pathways involving guanosine triphosphate–binding protein (G protein) activation, promote a signal transduction cascade leading to acrosomal exocytosis.

Whatever model of sperm-egg cell interaction is proposed must account for the fact that stimulation of human spermatozoa with a variety of stimuli, including ZP3, progesterone, PAF, and reactive oxygen species (*3, 5, 15*), results in the enhanced phosphorylation of a ZRK-like molecule. Thus, in addition to ZRK's role in ZP3 recognition, it appears to be a conduit for signal transduction after activation of the spermatozoa with several different types of agonist. It is possible that conception involves a primary adhesion event mediated by lectins and secondary interactions with multiple inducer-specific receptors flowing through a common redox-regulated tyrosine phosphorylation step, as recently suggested for the activation of NF-κB in T cells (*16*). Clearly, the complex cellular mechanisms that regulate sperm-egg interactions are still far from resolved. However, the new results will contribute directly to such fundamental objectives as the development of contraceptive vaccines and the diagnosis and treatment of male infertility.

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