PERSPECTIVES: DEVELOPMENT

How to Stimulate Your Partner

Anne M. Villeneuve

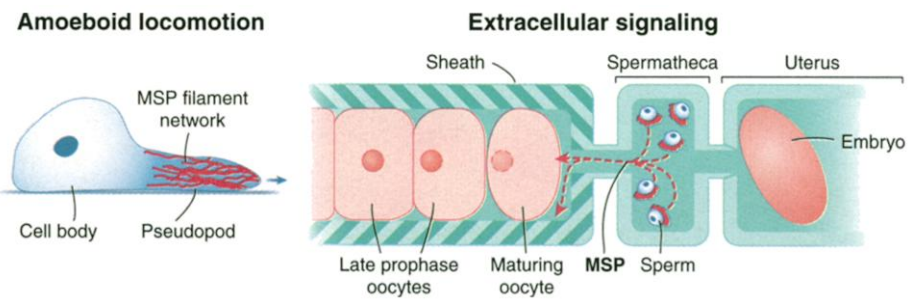
Animals have evolved a plethora of elaborate mechanisms to ensure successful sexual reproduction. These run the gamut from intricate mating rituals and release of pheromones, which aid in the identification of appropriate partners, to cell-cell signaling events that coordinate the timing of meiosis (cell division of egg and sperm precursors), gamete formation, and fertilization. The nematode *Caenorhabditis elegans*, an unrepentant minimalist of the animal kingdom, maximizes the efficiency of its reproductive resources in several ways. For example, its oocytes are arrested in meiotic prophase (like the oocytes of most animals) and do not mature until sperm become available (*1*). The presence of sperm triggers the oocytes to complete meiosis and stimulates ovulation by promoting contraction of the smooth muscle-like sheath that encases the developing oocytes (see the figure) (*2*). On page 2144 of this issue, Miller *et al.* (*3*) identify the signal from sperm that elicits these responses, and in so doing, they define an entirely new class of extracellular signaling molecules.

In a bold departure from the genetic strategies traditionally used to dissect biological circuitry in *C. elegans*, Miller *et al.* took a biochemical approach to isolating the maturation- and ovulation-stimulating signals emanating from sperm. They developed a bioassay in which sperm-conditioned medium was introduced into the reproductive tract of spermless females, and used this assay to purify a protein activity that could mimic the presence of sperm in stimulating oocyte maturation and gonad sheath contraction.

The authors isolated a signaling protein that turned out to be a well-known component of nematode sperm, the major sperm cytoskeletal protein (MSP). This protein has been implicated in the mechanics of sperm locomotion, playing a role analogous to that of the cytoskeletal protein actin (*4*). MSP constitutes about 15% of the total protein in nematode sperm (*5*), which are amoeboid cells that crawl along a solid substrate by means of a flattened extension called a pseudopod (see the figure). The pseudopod of *C. elegans* sperm looks and acts like the actin-rich pseudopodia and lamellipodia of crawling cells ranging from the slime mold *Dictyostelium* to mammalian white blood cells. These amoeboid sperm exhibit the characteristic membrane protrusion, ruffling, and cytoskeletal flow seen in actin-based motility, but they use a filament system based on MSP rather than actin.

Even though actin and MSP lack similarity in their amino acid sequences, there are remarkable parallels between actin-based and MSP-based locomotion (*6*). In particular, in both systems protrusive activity coincides with localized assembly and bundling of filament networks. Indeed, a reconstituted in vitro system that preserves the key elements of the motile apparatus of nematode sperm provided some of the first direct evidence in support of the now widely accepted model that filament assembly and bundling can generate the force to move cell membranes (*4*). Thus, in a very real sense, the discovery of Miller *et al.* is the moral equivalent of identifying actin as an extracellular signaling molecule.

Identifying a highly abundant intracellular structural protein as the major constituent of the signaling-active fractions of sperm-conditioned medium naturally raises concerns over whether MSP might have been a contaminant rather than the true signaling activity. Miller *et al.* alleviate such concerns by demonstrating that recombinant MSP expressed in bacteria elicits the same responses as MSP purified from sperm or sperm-conditioned medium. Moreover, part of the MSP-triggered response in oocytes is phosphorylation of the signaling molecule mitogen-activated protein kinase. This indicates that MSP, the founding member of a new class of signaling molecules, impinges on a highly conserved signal transduction cascade. Additional evidence that MSP is likely to be the bona fide endogenous signal directing oocyte maturation and sheath contraction comes from experiments showing that antibodies to MSP reduce ovulation and maturation rates when sperm are present. Miller *et al.*'s biochemical strategy for identifying the sperm-derived signal turned out to be an inspired choice because the *C. elegans* genome has about 40 genes encoding MSP (*7*) and so genetic redundancy would have thwarted efforts to identify this signal through mutational analysis.



The dual nature of an actin analog. (Left) The cytoskeletal protein MSP, an analog of actin, is necessary for the motility of the nematode's amoeboid sperm. The forward locomotion of amoeboid sperm is driven by localized polymerization and bundling of MSP filaments at the leading edge of the pseudopod. **(Right)** MSP also serves as an extracellular bipartite signaling molecule that triggers oocyte maturation (nuclear envelope breakdown, cortical rearrangement, and spindle assembly) as well as contraction of the gonad sheath cells that encase the developing oocytes. This contraction propels the maturing female gametes into the spermatheca and uterus (ovulation).

But the surprises continue! In attempting to define the portion of MSP responsible for its signaling activity, Miller and colleagues discovered that the two nonoverlapping fragments of MSP elicited reciprocal responses. A 21–amino acid carboxyl-terminal fragment was fully potent in stimulating contraction of sheath cells but did not stimulate oocyte maturation, whereas the amino-terminal 106–amino acid fragment elicited a robust oocyte maturation response but did not stimulate sheath contraction. Thus, MSP apparently contains not one but two separate signaling moieties, possibly activating distinct signal transduction pathways in oocytes and gonad sheath cells. These experiments further indicate that although filament formation is intrinsic to MSP's orchestration of sperm motility, it is unlikely to be required for either of its signaling activities. Previous structure-func-

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tion studies predicted that neither of the signaling-proficient MSP fragments should be able to form filaments (*8*).

How does MSP get released by sperm so that it can carry out its dual signaling task? The standard secretion pathway coupled to protein synthesis does not appear to be an option, because MSP lacks a signal sequence and the sperm lack ribosomes, endoplasmic reticulum, and Golgi apparatus (*9*). Nematode spermatids have an alternative apparatus for delivering prepackaged glycoproteins to the cell surface during spermiogenesis, but this also seems an unlikely route, because mutant spermatocytes that never undergo spermiogenesis are nevertheless signaling competent (*2*). The signaling competence of spermatocytes further indicates that sperm motility and pseudopod formation are also dispensable for signal delivery.

The discovery that a major intracellular cytoskeletal component known to be involved in sperm motility can also behave as an extracellular bipartite signaling molecule is unanticipated and provocative. Because proteins containing a conserved amino-terminal MSP-like domain are found in fungi, plants, and animals, it is possible that MSP-related signaling events may be identified in other phyla as well. Regardless of whether this turns out to be the case, however, the saga of nematode MSP should serve as a poignant cautionary tale for biologists everywhere: Other proteins that we think we know extremely well may turn out to be leading dual lives!

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PERSPECTIVES: GEOLOGY

The Deadliest Intraplate Earthquake

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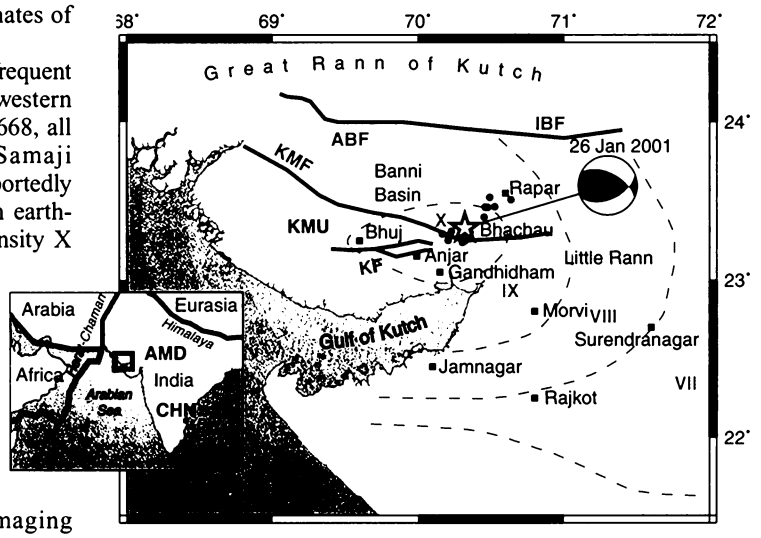
A catastrophic earthquake of magnitude 8 struck the Bhuj-Anjar-Bhachau region of Kutch, Gujarat, in western India on the morning of 26 January 2001 (*1*). The earthquake epicenter (see the figure) was located at 23.326°N, 70.317°E, and its focal depth was up to 23 km. A conservative official estimate puts the number of human lives lost at 30,000 and the economic loss at U.S. $10 billion. News media estimates of the human lives lost exceed 50,000.

In the historic past, large but infrequent earthquakes have occurred in the western part of the Kutch region. In May 1668, all 30,000 houses of the town of Samaji (25°N, 68°E) on the Indus delta reportedly sank into the ground because of an earthquake with maximum damage intensity X on the 12-point Modified Mercalli (MM) scale. An earthquake of magnitude 8 occurred in the Great Rann of Kutch on 16 June 1819, forming a 90-km-long scarp with a height of up to 9 m. It came to be known locally as "Allah Bund" or "Wall of God." The earthquake claimed 1500 lives in Kutch and 500 in Ahmedabad. The last damaging earthquake in the region, the magnitude 7 Anjar earthquake of 21 July 1956, caused 115 deaths.

The epicenter of the 2001 Bhuj earthquake is located about 15 km northwest of Bhachau and 60 km east of Bhuj. The maximum damage was of MM intensity X in an area of 100 km by 60 km (see the figure). Total collapse of nonengineered houses and ground cracks of up to 1-m width were witnessed in the epicentral region. As expected, damage in river floodplains was much worse than in hard rock areas. In Ahmedabad, 250 km from the epicenter, many highrise buildings collapsed, possibly because of faulty design. In Anjar, Bhachau, Gandhidham, and Ahmedabad, multistoried buildings sank up to one floor into the ground, possibly because of a quicksandlike effect in dry sandy soil areas. The earthquake caused widespread liquefaction in the Great Rann saline-marshy lowlands to the north and the Little Rann to the southeast. As a result of soil liquefaction and subsidence, railway lines were heavily damaged, as were several small and medium-sized dams. The earthquake was felt as far away as Chennai on the southeastern coast of India.

The Harvard focal mechanism solution of the Bhuj earthquake indicates a thrust fault with strike of 65°, dip of 50°, and slip of 50°. The maximum dislocation, for a southward dipping fault plane, is estimated as about 8.5 m (*2*). Depth estimates vary from 10 to 23 km. The earthquake was followed by more than 100 aftershock events of magnitude above 4, including 10 events of magnitude above 5. Focal mechanism solutions of a few of the major aftershocks also indicate a thrust environment.

The epicenter of the main shock lies close to the eastern part of the Kutch Mainland Fault (*3*) (see the figure). The earthquake occurred about 400 km east of the Herat-Chaman plate boundary and more than 1000 km south of



Map of the 2001 Bhuj earthquake. The epicenter is marked with a star. Isoseismals in MM intensity scale (Roman numerals) are shown as green broken lines. The epicenters of a few aftershocks, determined with data from a local network set up by the National Geophysical Research Institute of India, are shown as blue dots. KMU, Kutch Mainland Uplift. Major faults (red lines): ABF, Allah Bund Fault; IBF, Island Belt Fault; KMF, Kutch Mainland Fault; KF, Katrol Fault. (**Inset**) The Indian plate boundaries (dark blue lines) form a triple junction northwest of the Kutch region. AMD, Ahmedabad; CHN, Chennai.

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