

Rules of Evidence: Calcium ions and fertilization

Biology, like all science, does not deal with Facts; rather, it deals with evidence. Several types of evidence are presented in this book, and they are not equivalent in strength. In Chapter 1, we discussed the "Find it/Lose it/Move it strategy. Here, we'll see how this was used in determining that calcium was critically important in the activation of sea urchin eggs.

Find it: Correlative evidence

The first, and weakest, type of evidence is correlative evidence. Here, we find correlations between two or more events and then make the inference that one event causes the other. For example, upon the meeting of sea urchin sperm and egg, a wave of free Ca^{2+} spreads across the egg, and this wave of Ca^{2+} is thought to activate the egg. This chain of events has been shown in several ways, most convincingly by aequorin fluorescence (Steinhardt et al. 1977; Shimomura 1995; Steinhardt 2006).

Although one might infer that the meeting of egg and sperm caused the Ca^{2+} wave, and that this Ca^{2+} wave caused egg activation, such a correlation of events with one another does not necessarily demonstrate a causal relationship. It is possible the meeting of gametes first caused the flow of Ca^{2+} across the egg and then, separately and by some other mechanism, activated the egg. It is also conceivable that some aspect of egg activation caused the Ca^{2+} release. The correlated occurrences of these events could even be coincidental and have no relationship to one another.* Correlative evidence provides a starting point for many investigations, but one cannot say that one event causes another based solely on correlation.

Lose it: Loss-of Function evidence

The next type of evidence is called loss-of-function evidence, also known as negative inference evidence. Here, the absence of the postulated cause is associated with the absence of the effect. While stronger than correlative evidence, loss-of-function evidence still does not exclude other explanations. For instance, when calcium chelators such as EDTA were injected into the egg prior to fertilization, released Ca^{2+} failed to activate the egg. This would imply that Ca^{2+} is necessary for egg activation. However, data from such inhibitory studies (including studies from loss-of-function mutations) always leave open the possibility that the inhibitor suppresses more than just the process being studied. For instance, when protease inhibitors caused the failure of mammalian fertilization, it was assumed that these inhibitors were blocking the action of proteases released from the acrosome. As a result, biologists thought that the mammalian acrosome releases soluble proteases that digest the zona. Later experiments, however, demonstrated that the protease inhibitors inhibited the acrosome reaction itself so that the proteases were never released (Llanos et al. 1993). Thus, one couldn't tell whether soluble proteases played any role in mammalian fertilization.

Move it: Gain-of-Function evidence

The strongest type of evidence is gain-of-function evidence. Here the initiation of the first event causes the second event to happen even in instances where or when neither event usually occurs. Thus, when calcium ionophores (which can shuttle Ca^{2+} across membranes) were added

to *unfertilized* eggs, Ca^{2+} was released from intracellular storage and the eggs became activated even without fertilization (Steinhardt and Epel 1974).

Progression of evidence and coherence

Correlative (“find it”), loss-of-function (“lose it”), and gain-of-function (“move it”) evidence must consistently support each other to establish and solidify a conclusion. This progression of “find it, lose it, move it” evidence is at the core of nearly all studies of developmental mechanism (Adams 2000). Sometimes it can be found in a single paper, and sometimes, as the case above illustrates, the evidence comes from many laboratories. “Every scientist,” writes Fleck (1979), “knows just how little a single experiment can prove or convince.” Rather, “an entire system of experiments and controls is needed.” Science is a communal endeavor, and it is doubtful that any great discovery is the achievement of a single experiment, or of any individual.

Science also accepts evidence better when it fits into a system of other findings. This is often called coherence. For instance, the ability of calcium to activate the egg became a standard part of fertilization physiology when Ca^{2+} was shown to cause both the resumption of cell division and the initiation of translation—two separate components of egg activation. Also, once the sperm was found to activate phospholipase C—the enzyme that synthesizes IP_3 —and IP_3 was found to activate intracellular calcium release in numerous cells, the release of Ca^{2+} became understood as being the central element of sea urchin egg activation. It fit into a much wider picture of physiological calcium release, and the mechanisms for its synthesis and its effects all fit together.

*In a tongue-in-cheek letter spoofing such correlative evidence, Sies (1988) demonstrated a remarkably good correlation between the number of storks seen in West Germany from 1965 to 1980 and the number of babies born during those same years. Any cause-and-effect scenario between storks and babies, however, would certainly fly in the face of the evidence presented in this chapter.

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