<Further Development 7.7>

The IP_3 Pathway Activates the Egg

In sea urchin eggs, IP_3 is formed initially at the site of sperm entry and can be detected within seconds of sperm-egg attachment. Inhibiting IP_3 synthesis prevents Ca^{2+} release (Lee and Shen 1998; Carroll et al. 2000), whereas injected IP_3 can release sequestered Ca^{2+} and lead to cortical granule exocytosis (Whitaker and Irvine 1984; Busa et al. 1985). Moreover, these IP_3-mediated effects can be thwarted by pre-injecting the egg with calcium-chelating agents (Turner et al. 1986).

IP_3-responsive calcium channels have been found in the egg endoplasmic reticulum. The IP_3 formed at the site of sperm entry is thought to bind to IP_3 receptors in these calcium channels, effecting a local release of Ca^{2+} (Ferris et al. 1989; Furuichi et al. 1989). Once released, Ca^{2+} can diffuse directly, or it can facilitate the release of more Ca^{2+} by binding to *calcium-triggered calcium-release receptors*, also located in the cortical endoplasmic reticulum (McPherson et al. 1992). These receptors release stored Ca^{2+} when they bind Ca^{2+}, so binding Ca^{2+} releases more Ca^{2+}, which binds to more receptors, and so on. The resulting wave of calcium release is propagated throughout the cell, starting at the point of sperm entry. The cortical granules, which fuse with the cell membrane in the presence of high Ca^{2+} concentrations, respond with a wave of exocytosis that follows the

calcium wave. Mohri and colleagues (1995) have shown that IP_3-released Ca^{2+} is both necessary and sufficient for initiating the wave of calcium release.

PHOSPHOLIPASE C: THE GENERATOR OF IP_3 If IP_3 is necessary for Ca^{2+} release and phospholipase C is required in order to generate IP_3, the question then becomes, what activates PLC? This question has not been easy to address since (1) there are numerous types of PLC that (2) can be activated through different pathways, and (3) different species use different mechanisms to activate PLC. Results from studies of sea urchin eggs suggest that the active PLC in echinoderms is a member of the γ (gamma) family of PLCs (Carroll et al. 1997, 1999; Shearer et al. 1999). Inhibitors that specifically block PLCγ inhibit IP_3 production as well as Ca^{2+} release. Moreover, these inhibitors can be circumvented by microinjecting IP_3 into the egg. How PLCγ is activated by sperm is still a matter of controversy, although inhibitor studies have shown that membrane-bound kinases (Src kinases) and GTP-binding proteins play critical roles; Kinsey and Shen 2000; Giusti et al. 2003; Voronina and Wessel 2003, 2004; Townley et al. 2009). One possibility is that NAADP brought in by the sperm to initiate electrical depolarization also activates the enzyme cascade leading to IP_3 production and calcium release (Churchill et al. 2003; Morgan and Galione 2007).