Imaging the motility of inositol trisphosphate receptors in intact mammalian cells using single particle tracking photoactivated localization microscopy (sptPALM)

I.F. Smith, D. Swaminathan and I. Parker, Neurobiology and Behavior, University of California, Irvine, CA 92697, USA. (Introduced by Bradley Launikonis)

Inositol trisphosphate receptors (IP_3Rs) are Ca^{2+}-permeable channels in the membrane of the endoplasmic reticulum (ER) that liberate Ca^{2+} sequestered in ER stores to generate cytosolic Ca^{2+} signals that control diverse cellular functions including gene expression, secretion and synaptic plasticity (Berridge, Lipp & Bootman, 2000). These channels are gated by both the second messenger IP_3 and biphasically by Ca^{2+} itself. Activation of IP_3Rs by Ca^{2+} ions diffusing from neighboring channels thus results in a regenerative amplification by Ca^{2+}-induced Ca^{2+} release (CICR). The extent of this functional coupling depends strongly upon the spacing between IP_3Rs, so that spatial localization of these channels is a major determinant of cellular Ca^{2+} signals. In particular, Ca^{2+} imaging studies in numerous cell lines and in *Xenopus* oocytes reveal local IP_3-mediated Ca^{2+} signals ("puffs") that arise through the concerted opening of several IP_3R channels within tight clusters.

The mechanisms underlying the aggregation and maintenance of IP_3Rs within these clusters are controversial. Puffs arise at just a few, fixed locations within a cell, suggesting that the clusters are relatively stable entities; and calcium blips generated by lone IP_3Rs are similarly immotile (Smith & Parker, 2009; Smith *et al.*, 2009). In contrast, imaging studies employing GFP-tagged or immunostained IP_3Rs show a dense distribution throughout the cell. Moreover, the majority IP_3Rs can diffuse freely within the ER membrane, and aggregate into clusters following sustained (minutes) activation of IP_3 signaling and/or cytosolic Ca^{2+} elevation, or even undergo clustering in response to IP_3 within just a few seconds (Taufiq-Ur-Rahman *et al.*, 2009).

These apparently different behaviors may be explained because Ca^{2+} imaging studies detect only functional IP_3Rs (those that mediate Ca^{2+} liberation from the ER), whereas imaging studies utilizing immunostaining or GFP-tagged IP_3Rs report on the behavior of the entire population of IP_3R proteins. We therefore hypothesized that a majority of IP_3Rs are motile, but are either functionally unresponsive or mediate Ca^{2+} liberation only during sustained global elevations of cytosolic $[Ca^{2+}]$. Local Ca^{2+} signals arise, instead, from a small subset of IP_3Rs that are anchored, individually or in clusters, by association with static cytoskeletal structures and which, possibly as a consequence of this anchoring, display high sensitivity to IP_3 to generate Ca^{2+} blips and puffs (Parker & Smith, 2010).

In order to test this hypothesis we have utilized the new generation of photoactivatable genetically encoded proteins to track the motility of thousands of individual IP_3R molecules with nanoscale spatial resolution and millisecond temporal resolution (sptPALM) (Manley *et al.*, 2008). We find that IP_3Rs can be distinguished into two groups with relatively high or low motility and are currently investigating whether there is a spatial correlation to the differences in observed motilities.

Berridge MJ, Lipp P, Bootman MD. (2000) *Nature Reviews Molecular Cell Biology* **1:** 11-21.

Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, Lippincott-Schwartz J. (2008) *Nature Methods* **5:** 155-157.

Parker I, Smith IF. (2010) *Journal of General Physiology* **136:** 119-127.

Smith IF, Parker I. (2009) *Proceedings of the National Academy of Sciences USA* **106:** 6404-6409.

Smith IF, Wiltgen SM, Shuai J, Parker I. (2009) *Science Signaling* **2:** ra77.

Taufiq-Ur-Rahman, Skupin A, Falcke M, Taylor CW. (2009) *Nature* **458:** 655-659.