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An inside job: Annexin 1A-Inositol 1,4,5-trisphosphate receptor interaction conveys endoplasmic reticulum luminal Ca^{2+} sensitivity

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ABSTRACT

Cytoplasmic Ca^{2+} is a pivotal regulator of IP_3R activity. It is however controversial whether the $[Ca^{2+}]$ in the Endoplasmic Reticulum lumen also directly regulates channel function. We highlight a recent paper that demonstrates that luminal $[Ca^{2+}]$ potently inhibits IP_3R activity. This regulation occurs indirectly by an interaction mediated through a binding partner, likely Annexin 1A.

Inositol 1,4,5-trisphosphate receptor (IP_3R) activity is subject to fine-tuning by a myriad of factors which contribute to the versatility of Ca^{2+} signaling and ultimately allow the control of diverse physiological endpoints with exquisite fidelity [1]. IP_3R regulation is accomplished via post translational modifications, interactions with an array of protein binding partners, ATP, and most importantly, Ca^{2+} itself [2,3]. Although regulation by cytosolic Ca^{2+} is well established, whether IP_3R activity is influenced by the $[Ca^{2+}]$ in the ER lumen and if regulation is direct, or is mediated through a binding partner is not established [4]. While studies have shown that depletion of ER stores results in attenuated Ca^{2+} release, the current literature in support of ER regulation of IP_3R remains equivocal as previous studies directly monitoring IP_3R single channel activity have failed to report luminal regulation and more indirect studies of Ca^{2+} release are complicated by not directly measuring the luminal and cytoplasmic $[Ca^{2+}]$ [5].

A recent study published in eLife re-examines luminal regulation of IP_3R activity and reports that high ER luminal $[Ca^{2+}]$ markedly inhibits IP_3R channel activity measured at the single channel level [6]. This regulation was not apparent in earlier studies because using the "on-nucleus" configuration of the patch-clamp technique, with common pipette and bath solutions, the ER passively depletes as a result of the absence of SERCA pump activity. In the current study, in addition to maintaining the ER luminal $[Ca^{2+}]$ by addition of Mg-ATP to fuel SERCA, rigorous Ca^{2+} buffering in the cytoplasm (bath) eliminated any effects of Ca^{2+} flowing through the pore and acting on Ca^{2+} binding sites on cytoplasmic portions of the IP_3R. Notably, inhibition of IP_3R channel activity was lost when patches were excised from the nucleus to expose the luminal face of the ER membrane to low $[Ca^{2+}]$. This observation taken together with the lack of direct regulation of IP_3R channel activity by luminal Ca^{2+}, strongly indicates that IP_3R regulation occurred indirectly through the interaction with a rather loosely associated Ca^{2+} sensing binding partner.

In search of the luminal binding partner, the authors rationalized that the protein would interact with conserved regions of the short intra ER luminal loops present between transmembrane helices in IP_3R. Based on the observation that synthetic peptides mimicking the second luminal loop (L2P) disrupted luminal regulation, this region was used as bait for pull-down assays with Mass Spectrometry analysis. This work flow identified the Ca^{2+} and phospholipid binding protein, Annexin A1 (ANXA1) as a promising candidate. Importantly, while other annexins were also captured by the pull down, only ANXA1 appeared to influence channel activity and knockdown of endogenous ANXA1 enhanced cytoplasmic Ca^{2+} signals in HEK and Hela cells. The interaction of IP_3R and ANXA1 to modulate channel activity appears to be a common, conserved mechanism because ANXA1 expression is widespread and inhibition of IP_3R by this mechanism occurred in a variety of cell lines representing predominant expression of different IP_3R subtypes. While the extent of inhibition of channel activity did not appear markedly different in these expression systems, further work will be necessary to define whether individual IP_3R subtypes are modulated in a distinct manner, mirroring their distinct regulation by cytoplasmic Ca^{2+} [7]. While the weight of evidence suggests that the ANXA1-IP_3R interaction may represent a common regulatory mechanism, some questions remain based on the inability to co-IP ANXA1 and IP_3R despite pull down with L2P and the inconclusive immunolocalization of ANXA1 in the ER lumen. Other techniques may be necessary to confirm what is likely a dynamic, transient, low affinity interaction between a minority of ANXA1 and possibly a specialized sub-population of expressed IP_3R.

IP_3R appear to be held in an inactive state by interaction with ANXA1 when ER Ca^{2+} stores are replete and this may provide a

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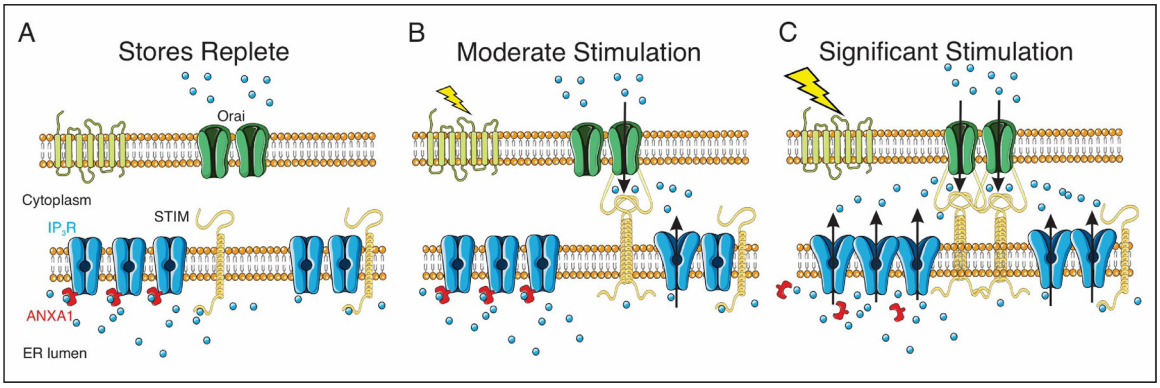

Fig. 1. In the resting state prior to a stimulus, it is likely that a majority of the IP_3R are held in an inactive state by the calcium binding protein ANXA1. The remaining IP_3R that respond to a low threshold stimulus are "licensed" to open either due to the presence of a licensing protein or are not associated with ANXA1 (A). This low threshold stimulus causes moderate, local depletion of the ER which presumably is not significant enough to cause ANXA1 dissociation (B). However, in the case of a strong stimulus, more significant store depletion causes IP_3R which previously were bound to ANXA1 to become active after the dissociation of the inhibitory protein, resulting in the recruitment of a greater amount of SOCE machinery (C).

mechanism to explain recent observations that suggest that a majority of IP_3R in cells are refractory to opening upon an increase in $[IP_3]$ [8,9]. The minority of IP_3R that readily respond to raising IP_3 are present in immobile clusters and are often localized at junctions between the ER and plasma membrane that express STIM and Orai proteins constituting the store operated Ca^{2+} entry (SOCE) machinery. The IP_3R that serve as the trigger for the initial response may either interact with an additional factor which "licenses" them, facilitating opening, or alternatively, are IP_3R not interacting with ANXA1 (Fig. 1 A). Activation of licensed IP_3R also would be predicted to locally deplete the ER and initiate SOCE. Minor or transient depletion at low stimulus thresholds, may not deplete the ER sufficiently to allow ANXA1 to dissociate from IP_3R and thus these IP_3R could represent a "strategic reserve" poised to be recruited following a strong stimulus which results in significant depletion of the ER and subsequent activation of STIM1 (Fig. 1 B). Activation of this new pool of responsive IP_3R could be envisioned to provide amplification to further deplete ER stores and recruit additional STIM1 to provide a persistent depletion and maintain Ca^{2+} influx at the PM for physiological processes that require sustained Ca^{2+} flux (Fig. 1 C). In some circumstances sustained activation of Ca^{2+} entry is detrimental and it is tempting to speculate that the IP_3R-ANXA1 interaction may protect cells from Ca^{2+} overload in these situations [10]. Future work will establish the consequences of the interaction between ANXA1 and IP_3R for Ca^{2+} homeostasis in the numerous native cell systems where IP_3 centric signaling is important for function and where dysregulation results in pathology.

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