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The Making and Breaking of Inositol 1,4,5-Trisphosphate Receptor Tetramers

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Abstract

Mammalian cells express three highly conserved inositol 1,4,5-trisphosphate (IP₃) receptor types (IP₃R1, IP₃R2 and IP₃R3), which have broadly similar characteristics, but markedly different distributions, and form homo- or heterotetrameric Ca²⁺ channels in endoplasmic reticulum (ER) membranes. A vast array of published work details how mature, ER membrane-located IP₃ receptor tetramers are regulated, but much less attention has been paid to the intriguing questions of how the tetramers are assembled and destroyed as part of their natural life cycle. Are they assembled at the ER membrane from nascent, or completely translated polypeptides? How are they disassembled and degraded? These questions and other recently defined modes of IP₃ receptor processing will be briefly reviewed.

Keywords

IP₃ Receptor; Calcium; Ubiquitin; Proteasome; Tetramer; Ion Channel

IP₃ RECEPTOR TETRAMER (tIP₃R) STRUCTURE AND ASSEMBLY

The last several years has seen a revolution in our understanding of tIP₃R structure, spearheaded by Serysheva's group (Fan et al., 2015; Baker et al., 2017). The current picture is that the four, ~2,700 amino acid subunits meld together into a mushroom shaped complex, with the 24 transmembrane (TM) domains (6 from each subunit) forming the base that spans the ER membrane (Fig. 1). Contact points between the individual subunits are extensive and the tetramers are very stable, as judged from the maintained integrity of homo- and heterotetramers after detergent solubilization and purification (Fan et al., 2015; Wojcikiewicz and He, 1995; Monkawa et al., 1995). Contact is particularly evident in the plane of the ER membrane, where TM domains 5 and 6 of each subunit interdigitate to form the channel pore, and in the cytosol, where the receptor C-terminal domains (CTDs) wrap around each other (Fig. 1); together these regions form the central core of tIP₃R that constitutes the IP₃-regulated Ca²⁺ conduction pathway (Fan et al., 2015; Baker et al., 2017). But how is this elegant tetrameric assemblage attained? Do individual, completely-translated IP₃R polypeptides spontaneously associate after they are released into the ER membrane, or is the assembly coor-dinated as nascent polypeptides emerge from ribosomes? This issue is complicated by the fact that the first TM domain of IP₃Rs (the first signal sequence that

would locate nascent IP₃R to the ER membrane) lies ~2250 amino acids away from the N-terminus (Fan et al., 2015; Pantazaka and Taylor, 2010). Further, one can assume that nascent IP₃Rs in various stages of completion will be synthesized from IP₃R mRNA by polyribosomes located adjacent to the ER membrane, with emerging TM domains inserted and released into the ER membrane via the translocon (Wells et al., 2015; Deutsch, 2003). Given the likely spacing of ribosomes on mRNA (Viero et al., 2015), it is probable that nascent IP₃Rs with incompletely formed TM and CTD regions will have the opportunity to associate with more completely formed nascent receptors. The details of how this works for tIP₃R is not yet defined experimentally, and remarkably very little is known about this for other oligomeric ion channels (Liu et al., 2016; Gan et al., 2015).

In contrast, quite a bit is known about the synthesis and ER membrane insertion of individual IP₃R subunits (Joseph et al., 1997; Galvan et al., 1999; Pantazaka and Taylor, 2010). In particular, it is clear that TM domain 1 is sufficient to initiate ER membrane localization, so long as 26–34 residues beyond its end are translated (a region that includes the TM domain 2), presumably so that TM domain 1 is sufficiently exposed to be recognized by the signal recognition particle (Pantazaka and Taylor, 2010). ER membrane insertion is also critical for tetramerization, since constructs containing only TM domains oligomerize (Joseph et al., 1997; Galvan et al., 1999), and conversely, IP₃ receptor constructs without TM domains do not oligomerize (Galvan et al., 1999). Thus, although the different subunits in a mature tIP₃R interact at many points in the first ~2250 amino acids (Fan et al., 2015; Baker et al., 2017), those regions alone are insufficient to drive oligomerization. This has implications for tIP₃R synthesis, as it suggests that nascent IP₃R polypeptides will not interact until TM domains emerge.

An interesting wrinkle regarding this topic are data obtained using concatameric IP₃Rs, in which IP₃Rs are linked tail-to-head; i.e., the C-terminus of one IP₃R polypeptide (“A”) is linked to the N-terminus of the next IP₃R polypeptide (“B”) via a short amino acid linker (Alzayady et al., 2013; Alzayady et al., 2016). There is reason to expect that this approach would yield functional oligomers, because fortuitously the C- and N-termini of adjacent subunits in a tetramer are very close (Fig. 1; Fan et al., 2015). This approach has been extended to create a four subunit tIP₃R concatamer ABCD, that can yield important information on tIP₃R regulation (Alzayady et al., 2016). The functionality of such channels shows that emerging TM regions of a newly synthesized IP₃R polypeptide (e.g., B) can associate efficiently with the fully-formed 6 TM region bundle of a previous IP₃R polypeptide (e.g., A). This argues in favor of the idea that completely-translated, fully-formed IP₃ receptor subunits can associate, and against a requirement for co-translational assembly of nascent polypeptides in the creation of tIP₃Rs.

Overall, while it is clear that the first TM region of IP₃R specifies membrane localization and assembly, there is still a long way to go before we understand how the four subunits in a tetramer become “locked” together in a stable, yet flexible complex.

tIP₃R DEGRADATION

All cellular proteins have a finite lifetime and are turned over; i.e. are selected for disposal and degraded to their constituent amino acids (Ciechanover, 2017). tIP₃Rs are no exception and have a half-life of ~10 h (Wojcikiewicz, 1995; Khan and Joseph, 2003), although very little is known about the mechanism of basal tIP₃R turnover; i.e., how the tetramers are deconstructed and degraded (Wright and Wojcikiewicz, 2016). What little is known has come from studies on tIP₃R “down-regulation,” the process by which activated tIP₃Rs in stimulated cells are degraded by the ubiquitin-proteasome pathway (UPP) (Wright and Wojcikiewicz, 2016). The UPP is a two-step process; first, ubiquitin chains are conjugated to substrates, and then, if the chain topology is correct, the ubiquitinated substrate is recognized by the proteasome and degraded (Komander and Rape, 2012, Ciechanover, 2017). The UPP is currently the focus of intense interest, since it is now known to be the major route of protein degradation in eukaryotic cells and mediates the selective destruction of many important proteins, including signaling pathway proteins and regulators of the cell cycle and transcription (Komander and Rape, 2012, Ciechanover, 2017). In addition, it is responsible for “quality control” in the ER; i.e., the selective degradation of misfolded nascent or mature proteins, and of unused subunits of multimeric proteins, in a process known as ER-associated degradation (ERAD) (Ruggiano et al., 2014; Printsev et al., 2016). That activated endogenous IP₃Rs (IP₃R1, IP₃R2 and IP₃R3) are targeted by the UPP (and in fact, the ERAD pathway) is remarkable. Electrophysiological studies show that tIP₃Rs alternate between open and closed states, with IP₃ increasing the proportion in the open conformation (Foskett et al., 2007). However, as this process occurs (e.g., during cell stimulation with an agonist that makes IP₃), a fraction of tIP₃Rs are targeted for ubiquitination and subsequent degradation by the proteasome (Wright and Wojcikiewicz, 2016). The resulting decline in cellular IP₃R levels reduces the sensitivity of ER Ca²⁺ stores to IP₃ (Wright and Wojcikiewicz, 2016). It occurs in a wide range of mammalian cell lines *in vitro*, in various primary cultures, in mouse oocytes after fertilization, and in rat pancreas *in vivo* (Wright and Wojcikiewicz, 2016). Thus, IP₃R down-regulation appears to be a widespread homeostatic process—cells adapt to persistent activation of IP₃-dependent signaling pathways by reducing the expression of the channels that respond to IP₃. This provides cells with a mechanism to limit increases in Ca²⁺ mobilization and may protect against deleterious effects of over-activation of Ca²⁺ signaling pathways, or may serve to oppose processes that depend upon IP₃R-induced Ca²⁺ mobilization from the ER (Wright and Wojcikiewicz, 2016). Importantly, IP₃R processing by the UPP also seems to be the mechanism by which IP₃Rs are turned-over under basal conditions, since deletion of proteins that mediate IP₃R ERAD increases basal IP₃R levels (Wright et al., 2015). Thus, IP₃R ERAD appears to be a key part of the IP₃R life cycle, whether it be the subtle recognition and turnover of tIP₃Rs under resting conditions, or the wholesale destruction of tIP₃Rs when cells are stimulated and open probability is substantially raised.

So what are the proteins that mediate tIP₃R ERAD? The keys players are (i) the erlin1/2 complex, an ~2MDa ER membrane complex composed of an assemblage of ~40 erlin1 and erlin2 molecules that binds directly to activated IP₃Rs and recruits the ubiquitin ligase RNF170 (Pearce et al., 2009, Lu et al., 2011), and (ii) the p97/Ufd1/Npl4 complex that binds

to ubiquitinated receptors and helps transfer them to the proteasome (Alzayady et al., 2005) (Fig. 1). Interestingly, while the p97/Ufd1/Npl4 complex is generic and mobilizes many substrates (Ruggiano et al., 2014; Printsev et al., 2016), the erlin1/2 complex-RNF170 module appears to be uniquely tailored to IP₃Rs and appears to be a specific complex for processing IP₃Rs (Wright and Wojcikiewicz, 2016). Remarkably, a point mutation in RNF170 (arginine¹⁹⁹ to cysteine) destabilizes the protein (Wright et al., 2015) and causes neurodegeneration that leads to autosomal dominant sensory ataxia (Valdmanis et al., 2011), and deletion of RNF170 in mice has similar effects (Kim et al., 2015). Also, mutations to erlin1 and, more commonly, erlin2 have been linked to neurodegenerative diseases (Wright and Wojcikiewicz, 2016; Novarino et al., 2014; Fink, 2013). For example, several mutations to the erlin2 gene, including point mutations leading to amino acid substitutions, are linked to hereditary spastic paraplegia (Yildirim et al., 2011; Alazami et al., 2011; Al-Saif et al., 2012; Wakil et al., 2013; Tian et al., 2016). Thus, perturbation of the erlin1/2 complex-RNF170 module causes neurodegeneration, most likely by interfering with normal tIP₃R processing. The key questions that remain are how exactly do activated tIP₃Rs and the erlin1/2 complex interact, how do mutations to the erlin1/2 complex affect function of the complex and tIP₃R ERAD, and how are tIP₃Rs disassembled.

NEW AND SURPRISING MODES OF IP₃R PROCESSING BY THE UPP

Two recent papers provide evidence for novel modes of IP₃R regulation by the UPP (Kuchay et al., 2017; Bononi et al., 2017). First, it has been shown that IP₃R3 can be targeted for ubiquitination and degradation by the FBXL2-containing SCF E3 ligase that is normally associated with degradation of proteins involved in cell cycle progression, and that this targeting of IP₃R3 is inhibited by the tumor suppressor TPEN (Kuchay et al., 2017). Because normal IP₃R3 levels appear to be required for Ca²⁺ signaling that supports appropriate cell death responses, excessive UPP-mediated degradation of IP₃R3 in TPEN-deficient cells may suppress Ca²⁺ signaling sufficiently to allow stressed and damaged cells to survive, enhancing oncogenesis and tumor survival. Second, IP₃R3 appears to be targeted by BAP-1, another tumor suppressor, that binds to, de-ubiquitinates and thus stabilizes IP₃R3 (Bononi et al., 2017). This cytosolic action of BAP-1, in addition to its nuclear effects, is disrupted when BAP-1 is mutated or deleted, increasing IP₃R3 ubiquitination and degradation, and again potentially enhancing oncogenesis and tumor survival. This intriguing work indicates that aberrant IP₃R processing can contribute to cancer progression and identifies IP₃R3 as a therapeutic target. Many questions remain, however. In particular, the conclusions are largely predicated on the assumption that IP₃R3, but not IP₃R1 or 2, has a key role in apoptotic Ca²⁺ signaling, but that is by no means certain (Ivanova et al., 2014).

FUTURE CHALLENGES

There is a conspicuous knowledge gap regarding how the four subunits of tIP₃R become interdigitated during synthesis. Filling this gap will advance our understanding of tIP₃Rs and ion channels in general, since so little is known about this topic. Likewise, very little is known about tIP₃R disassembly and IP₃R subunit retrotranslocation from the ER membrane. There is no evidence for IP₃Rs being translocated to the cytosol and IP₃R fragments do not appear during ERAD (Oberdorf et al., 1999; Sliter et al., 2008), suggesting that IP₃R

receptors undergoing degradation are very rapidly consumed as they are “pulled” from the ER membrane. Indeed, there is some evidence that non-tetrameric IP₃R oligomers form during tIP₃R ERAD (Sliter et al., 2008), indicating that individual subunits can be removed from tIP₃Rs. It appears that progress will be made when more is known about the suite of proteins, beyond the p97/Ufd1/Npl4 complex, that bind to tIP₃Rs once they have been recognized and ubiquitinated by the erlin1/2 complex-RNF170 module (Fig. 1). Finally, the novel FBXL2- and BAP1-mediated processing of IP₃R3 indicates that UPP-mediated degradation of tIP₃Rs is not limited to ERAD via the erlin1/2 complex-RNF170 module, and is more complex than originally thought (Wright and Wojcikiewicz, 2016). Thus, aberrant tIP₃R processing may contribute to both cancer (Kuchay et al., 2017; Bononi et al., 2017) and neurodegeneration (Egorova and Bezprozvanny, 2017).

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Biography

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Figure 1.

The key elements of tIP₃R ERAD. An tIP₃R is shown in the process of being targeted by an erlin1/2 complex (dark gray) and constitutively bound RNF170 (red, with the critical RING domain gold). Only 2 of the 4 subunits of the tIP₃R are depicted (colored green and red); each is ~2,700 amino acids in length and has 6 TM regions. RNF170 catalyzes tIP₃R ubiquitination, resulting in the attachment of homogenous chains of K48- or K63-linked ubiquitin (red ellipses) to cytosolic regions of tIP₃R (Wright and Wojcikiewicz, 2016). These chains are recognized by the p97/Ufd1/Npl4 complex that facilitates the transfer of proteins to the proteasome for degradation. Note that the structure shown is of an inactive (closed) tIP₃R and that the conformational changes that elicit channel opening, and presumably trigger the association of the erlin1/2 complex-RNF170 module with tIP₃R, have yet to be defined.