Targeting of Inositol 1,4,5-Trisphosphate Receptors to the Endoplasmic Reticulum by Multiple Signals within Their Transmembrane Domains*

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Most inositol 1,4,5-trisphosphate receptors (IP₃R) are expressed in the endoplasmic reticulum (ER), where their precise distribution underlies the spatially complex Ca²⁺ signals evoked by extracellular stimuli. The signals that target IP₃R to the ER or, less commonly, to other membranes are unknown. We expressed yellow fluorescent protein-tagged fragments of type 1 IP₃R alone or fused with a plasma membrane protein to establish the determinants of ER targeting in COS-7 cells. By using a combination of confocal imaging and glycoprotein analyses, we demonstrated that any pair of the six transmembrane domains (TMD) linked by a luminal loop retains the protein within the ER, and when attached to a plasma membrane protein (ICAM-1), prevents it from reaching the medial Golgi. TMD1 or TMD2 alone were accumulated in mitochondria, whereas TMD5 and TMD6 were retained in ER, but were unable to prevent ICAM from reaching the plasma membrane. We conclude that IP₃R are targeted to the ER membrane only after synthesis of TMDs 1 and 2, and that after co-translational insertion of the remaining TMDs, redundant retention signals present in any pair of TMD retain IP₃R in the ER.

Inositol 1,4,5-trisphosphate receptors (IP₃R) are intracellular Ca²⁺ channels that mediate the release of Ca²⁺ from intracellular stores evoked by receptors that stimulate IP₃ formation. Their close relatives, ryanodine receptors (RyR), are another family of intracellular Ca²⁺ channels with a more restricted distribution and different modes of regulation. There are three closely related subtypes (1–3) of mammalian IP₃R, and although they and their splice variants differ subtly in some aspects of their regulation (3), their structures are likely to be similar (4). All IP₃R are tetrameric, and each subunit, which may be identical or different within a receptor (3, 5, 6), has a cytosolic IP₃-binding domain toward the N-terminal (7), separated from a channel toward the C-terminal by a large “modulatory domain” (8). Topology predictions and direct evidence (9, 10) suggest the existence of six transmembrane domains (TMD) toward the C-terminal of each IP₃R subunit. The last two TMD, together with the intervening loop from each of the four subunits, form the pore (11). Although the transmembrane topology of RyR subunits is unresolved, they are known to have an even number of TMD (probably six or eight) (12) and, as with IP₃R, the last pair of TMD form the pore (13); both the N and C termini are cytosolic. For both IP₃R and RyR, the last pair of TMD, together with ~25 conserved residues lying ~35 residues downstream of them, are also major determinants of assembly of the tetrameric protein (10, 14, 15).

Most IP₃R are expressed in the ER membrane (16–19), but IP₃ has also been reported to stimulate Ca²⁺ release from the nuclear envelope (20–22), nucleoplasmic reticulum (23), Golgi (24), and secretory vesicles (25), although the latter has been challenged (26). IP₃R are also expressed in the plasma membrane of some cells (27–29), where they may mediate Ca²⁺ entry (30). Evidence that some IP₃R contain sialic acid (31) provides further evidence that they can progress at least as far as the trans-Golgi (32). Even within the ER, IP₃R are not uniformly distributed. The complex spatial organization of IP₃-evoked Ca²⁺ signals suggests that IP₃R (and RyR) form unevenly distributed clusters (33–36). IP₃R are concentrated in specific regions of some cells, for example, the apical pole of pancreatic acini (37), but are excluded from others, for example, the dendritic spines of hippocampal neurons, which express RyR but not IP₃R (17, 38). IP₃R associate with a bewildering array of additional proteins (39), and many of these associations are likely to be important in determining the subcellular distribution of IP₃R. An interaction between residues close to the N-terminal of the IP₃R and Homer, for example, can facilitate targeting of IP₃R to dendritic spines (38) and allows IP₃R to specifically associate with other signaling proteins in the plasma membrane, notably metabolic glutamate receptors (40) and TRPC proteins (41).

ER-resident proteins can maintain their location either because they never leave the ER (they may either lack export signals or express retention signals) or because they are actively retrieved from the Golgi. Soluble proteins (and a few type II membrane proteins) are retained within the ER by a C-terminal KDEL motif (42), but the mechanisms responsible for retaining membrane proteins (particularly multi-spanning membrane proteins; Ref. 43) are neither so well established nor as universal (44). A cytosolic di-lysine motif at the C-terminal (KKXX or more rarely KXXX) allows COP1-mediated retrieval of ER-resident membrane proteins (44, 45). Neither IP₃R nor RyR have appropriately positioned di-lysine motifs.
Cytosolic di-arginine motifs (RR or RXR) also mediate ER targeting; they are effective wherever they are expressed, although surrounding residues influence the strength of the signal (45). There are 11 such sites distributed between the two major cytosolic domains of IP₃R₁, but neither of the C-terminal sequences satisfies the criteria for the most effective retention signals (45). A cytosolic C-terminal sequence (YXXLXXR), similar to the tyrosine-based endocytic signal (46), can also mediate ER retention (47), but it is absent from IP₃Rs. TMDs can also serve as ER localization signals for membrane proteins of various topologies (48). TMDs with centrally placed polar residues (49) can interact with Rer1p, which allows ER retrieval from the cis Golgi in COPI vesicles. Short TMD (<17 residues) with hydrophilic residues down one face may also promote ER targeting (50, 51), possibly by a Rer1p-independent pathway (52). Finally, some proteins may be trapped within the ER because they form large oligomeric structures that are too large to move onward (53) and others by luminal signals that have yet to be defined (54). Different retention and retrieval signals are likely to collaborate to target native ER membrane proteins (47, 55).

Previous work established that IP₃Rs lacking the entire N-terminal cytosolic region were targeted to the ER (18, 56, 57). For RyR too, deletion of much of the N-terminal region failed to affect targeting to the ER and the final TMD (homologous with TMD₆ of IP₃R) was sufficient to retain a plasma membrane protein in the ER (58). Here, we demonstrate that IP₃R₁ contains redundant ER-targeting sequences. Any pair of TMD ensures effective targeting to, and retrieval within, ER membranes, and either TMD of the last pair is retained within the ER membrane, albeit less effectively than pairs of TMD. We discuss the relative effectiveness of different TMD in the context of known ER retention signals and the implications for selective targeting of IP₃R (and other intracellular Ca²⁺ channels) to different subcellular locations.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfections**—COS-7 cells were maintained in minimum essential medium supplemented with 5.6 mM glucose, 10% (v/v) fetal bovine serum, and 2 mM L-glutamine. Transfections were carried out using LipofectAMINE 2000 (Invitrogen) reagent according to the manufacturer’s instructions.

**Generation of Expression Constructs**—Full-length rat type 1 IP₃R (lacking the S₁ splice site) (59) and various fragments of it (see Fig. 1) were subcloned in-frame into the pC1-EYFP vector (Clontech). Site-directed mutagenesis was used to change a single residue in the pore region (VG₂₂⁵₅₋D₋VLR to VG₂₂⁵₅₋A₋VLR) of all fragments that included the TMD₅–₆ region to inactivate the channel (60). Site-directed mutagenesis was used to change a single residue in the pore region (VG₂₂⁵₅₋D₋VLR to VG₂₂⁵₅₋A₋VLR) of all fragments that included the TMD₅–₆ region to inactivate the channel (60). ICAM-EYFP and chimeras of EYFP-tagged IP₃R fragments with ICAM (see Fig. 1) were generated by PCR amplification from the pC1-EYFP clones and then ligated into a vector containing ICAM-1 (a gift from Dr Mark Davis, Stanford University; Ref. 61). The pC1-EYFP and pECFP-ER vectors were from Clontech.

**Confocal Imaging**—COS-7 cells were grown to near confluency on glass coverslips, transfectioned as above, and 20 h after transfection, they were washed with phosphate-buffered saline (PBS) and fixed with 3.5% paraformaldehyde in PBS for 20 min. After fixation, cells were perme-
abibilized with Triton X-100 (0.1%, 10 min) and blocked with 5% BSA in PBS for 10 min. A rabbit anti-calreticulin antiserum (Calbiochem, 1:100 in PBS with 5% BSA) was then added for 2.5 h at 20 °C, followed by overnight incubation at 4 °C. Cells were then washed three times with PBS and incubated with a goat anti-rabbit Cy5-coupled secondary antibody (Jackson ImmunoResearch, 1:500 in PBS with 5% BSA) for 1 h at 20 °C. After three washes with PBS and a rinse in distilled water, coverslips were mounted in 85% glycerol with 2.5% n-propyl gallate and stored at −20 °C. Two methods were used to identify mitochondria. Transfected cells were incubated in the normal culture medium supplemented with MitoTracker Red CMXRos (100 nM, 30 min at 37 °C, Molecular Probes) or they were co-transfected with pDsRed1-Mito (Clontech) and the usual YFP constructs. In both cases, cells were washed and fixed as above. All imaging was performed using a Bio-Rad Radiance 2000 scanning confocal microscope. Images were imported into Adobe Photoshop™ and adjusted to use the full range of pixel intensities.

### Western Blotting, Cross-linking, and Deglycosylation

Membranes were collected from transfected COS-7 cells 24 h after transfection. Cells were washed twice, scraped into ice-cold PBS containing a protease inhibitor mixture (Sigma), lysed by 30 passages through a 25-gauge needle, and the membranes were collected by centrifugation (30,000 × g) for 30 min. For cross-linking and deglycosylation experiments, washed cells (from a 35-mm dish) were collected directly into lysis medium (300 μl, 50 mM Tris, 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 1% Triton X-100, the protease inhibitor mixture, pH 7.4), sonicated for 3 min, and left on ice for 30 min. For cross-linking, 0.1 mM bis(sulfosuccinimidyl) suberate (Sigma) was added to samples (100 μl), and they were incubated at 20 °C. After 20 min, reactions were quenched by the addition of ammonium acetate (20 μl, final concentration = 170 mM). The susceptibility of proteins to endoglycosidase H (final activity 0.01 units/100 μl, Roche Applied Science) and N-glycosidase F (1 unit/100 μl, Roche Applied Science) was assessed by incubating samples at 37 °C for 4 h. Reactions were terminated by boiling in SDS sample buffer. Proteins were separated by 4–12% SDS-PAGE (Invitrogen), transferred to Immobilon membranes (Millipore), and the EYFP-tagged proteins were identified by using affinity-purified rabbit anti-GFP antibodies, which also recognize YFP and CFP (AbCam, 1:1000), and horse radish peroxidase-conjugated donkey anti-rabbit secondary antiserum (AbCam, 1:2000). Antibody binding was detected by Supersignal West Pico Chemiluminescent substrate (Perbio).

### RESULTS

**Targeting of IP₃Rs to ER by Pairs of TMD**—Western blotting and immunocytochemistry established that full-length IP₃R1 tagged at its N-terminal with YFP could be expressed in COS-7 cells; others have shown that this tagged IP₃R functions normally (57). Expression of fragments of the IP₃R that included the pore region (TMD5–6) perturbed the morphology of the cells. Because such fragments would be expected to form permanently open channels (11, 57), we expressed the same IP₃R fragments but with a point mutation (Asp2550 Ala) to disable the pore (60). The morphology of cells expressing the mutated fragments was indistinguishable from normal cells. In all subsequent experiments with fragments that included TMD5–6, IP₃R with the pore mutation were used. The fragments used (and their nomenclature) are shown in Fig. 1.

An antibody to calreticulin (a protein restricted to the ER
lumen; Ref. 62) and CFP-ER (CFP tagged with the signal sequence of calreticulin at its N terminus and KDEL at its C terminus) revealed a characteristic ER structure, with strong perinuclear fluorescence and a tubular network extending toward the cell periphery (Fig. 2, B and C). Expression of YFP alone produced a very different pattern that was clearly not coincident with calreticulin staining (Fig. 2A), confirming that YFP is not itself targeted to the ER. Full-length IP3R co-localized with both ER markers (Fig. 2, B and C). We cannot exclude the presence of IP3Rs in the Golgi and ERGIC, but there was no evidence for their selective concentration within either (not shown, and see below). These results confirm the prevailing view that most IP3 receptors in most cells are targeted to the ER (3).

Fig. 3A confirms earlier work (18, 56, 57) by demonstrating that the large N-terminal cytosolic portion of the IP3R is not required for targeting to the ER, but goes further by showing that the C-terminal tail, which includes putative ER-retention signals (see beginning of text) can also be deleted (Fig. 3B). The latter observation is consistent with a report that full-length IP3R1 truncated immediately after TMD6 targets to the ER (18). Our results establish, therefore, that the region including the six TMD is all that is required to retain IP3R in the ER. Indeed, any pair of TMD together with the linking luminal loop (TMD1–2, TMD3–4, TMD5–6) is sufficient to retain the protein in the ER (Fig. 3, C–E). After centrifugation of cell homogenates, IP3R fragments were detected only in the membrane pellet, whereas CFP-ER was distributed equally between supernatant and membrane fractions (Fig. 3F). The distribution of CFP-ER presumably reflects release of this soluble protein from ER damaged during homogenization. The clear difference between CFP-ER and IP3R fragments confirms that the latter were not trapped within the ER lumen but were incorporated into the ER membrane.

TMD of IP3R Retain a Plasma Membrane Protein in the ER—To establish whether IP3R-targeting sequences could redirect other membrane proteins to the ER, we attached them to a plasma membrane protein, ICAM-1, which is a glycoprotein
with a large extracellular N-terminal domain and a single TMD (63). Fig. 4A confirms the plasma membrane localization of YFP-ICAM, which is clearly very different from the distribution of calreticulin. Attaching the TMD fragments of the IP$_3$R that were targeted to the ER to the C-terminal of ICAM caused the chimeric proteins to be both inserted into membranes (Fig. 4F) and targeted to the ER (Fig. 4, B–E). We conclude that any pair of TMD from IP$_3$R1 linked by a luminal loop is sufficient to retain an integral plasma membrane protein in the ER membrane.

**IP$_3$R Retention Sequences Prevent IP$_3$R from Reaching the Medial Golgi**—Glycosylation of N-linked glycoproteins begins in the ER and continues in the Golgi, where attachment of a terminal N-acetylglucosamine in the medial Golgi renders the glycoprotein insensitive to endoglycosidase H (32, 64). Glycoproteins remain sensitive to N-glycosidase F throughout this processing. The sensitivity of glycoproteins to endoglycosidase H, therefore, reveals whether they have progressed to the medial Golgi.

There are only two glycosylation sites on rat IP$_3$R1 (Asn$^{2476}$ and Asn$^{2504}$) (9), and both are present in the luminal loop linking TMD5 and TMD6. Full-length IP$_3$R and all fragments that include TMD5–6 migrated on SDS-PAGE (usually as a doublet) with a molecular mass greater than that predicted from their primary sequences, and they were sensitive to treatment with endoglycosidase H (Fig. 5A). Earlier work had likewise established that full-length IP$_3$Rs were sensitive both to N-glycosidase F (9, 65) and endoglycosidase H (66). These results establish that neither full-length IP$_3$R nor fragments containing TMD5–6 progress to the medial Golgi.

The extracellular domain of ICAM contains eight N-glycosylation sites (63). ICAM migrated as two bands on SDS-PAGE. Most protein was in the higher molecular weight band, which was resistant to endoglycosidase H but sensitive to N-glycosidase F (Fig. 5, B and C). The upper band is the fully glycosylated protein that has passed through the medial Golgi, and the lower band, which was susceptible to both enzymes, is the core glycosylated protein. The fusions of IP$_3$R fragments with ICAM that caused the latter to be retained within the ER (Fig. 4) also caused the resulting glycoproteins to become equally sensitive to N-glycosidase F and endoglycosidase H (Fig. 5, B and C). We conclude that the ER-targeting sequences of the IP$_3$R are sufficient to prevent the IP$_3$R and ICAM from progressing to the medial Golgi.

**Targeting of IP$_3$R Fragments Containing a Single TMD**—The minimal IP$_3$R fragments so far shown to be retained within the ER include both a pair of TMD and the intervening luminal loop, which is largest for TMD5–6 (Fig. 4). Therefore, we examined whether single TMD were able to retain proteins in the ER. The results shown in Figs. 6, A and B demonstrate that either of TMD5 or TMD6 alone is sufficient to allow effective targeting and retention within the ER. However, when attached to ICAM, neither one is sufficient to prevent the chimeras from reaching the plasma membrane (Fig. 6, C–E) and from undergoing the glycoprotein modifications associated with the medial Golgi (Fig. 6F). We conclude that TMD5 and TMD6 each provide a weak signal sufficient to retain the fragment within the ER, but when alone, neither is sufficient to retain a plasma membrane protein. The latter contrasts with a recent study of RyR1 in which the final TMD (equivalent to TMD6 of IP$_3$R) was shown to retain ICAM in the ER (58).

Whereas TMD1–2 was clearly targeted to ER (Fig. 3C), the single TMD constructs (TMD1 and TMD2) had an entirely different distribution (Fig. 7A) and co-localized with two different mitochondrial markers (Fig. 7, B and C). Most mitochondrial proteins are imported after synthesis on cytosolic ribosomes by translocases guided by an N-terminal-cleavable signal sequence (positively charged amphipathic α-helix) or by poorly defined internal sequences (67, 68). Targeting of TMD1 and TMD2 to mitochondria suggests that the proteins are likely to have folded properly. Because IP$_3$R1 lacks a cleavable signal sequence (14), the first TMDs are thought to direct the nascent protein (via signal recognition particle, SRP, and the Sec61p complex) (69) to the ER to allow subsequent co-translational integration of the remaining TMDs. Our results suggest that neither TMD1 nor TMD2 alone is capable of fulfilling this function; both (at least in the absence of the normal N-terminal) are required for co-translational targeting to the ER. Our conclusion, that TMD1 alone does not provide an ER signal sequence, is consistent with earlier work suggesting that it is, at best, a poor targeting sequence. A protein comprising only TMD1 (and so presumably more hydrophobic than longer fragments) was inserted into microsomal membranes during translation in vitro (14), but full-length IP$_3$R1 truncated immediately after TMD1 (and so, more hydrophilic than our construct) was largely cytosolic (10). The significance of these observations is discussed below.

**IP$_3$R Fragments Retained in the ER Are Neither Mis-folded Nor Complexed with Native IP$_3$R**—Proteins that fold improperly are initially retained in the rough ER by chaperones. It is important, therefore, to ask whether the presence of expressed proteins in the ER (Figs. 2–6) reflects the involvement of specific targeting sequences or their retention in the ER because of improper folding. Several independent lines of evidence argue strongly against the latter.

Our analyses depend upon the proper folding of the YFP used to report the location of each protein: the N-terminal of each IP$_3$R fragment and the central portion of each ICAM-IP$_3$R chimera (Fig. 1) must, therefore, have folded properly. Improperly folded proteins are retro-translocated from the ER and
then degraded in the cytosol by proteasomes (70), but in Western blots, there was no evidence for proteolysis of any of the expressed proteins (Figs. 3F, 4F, 5, and 6E). We would expect misfolded proteins to aggregate and/or associate with luminal chaperones. Yet, in fluorescence recovery after photobleaching experiments, there was almost complete (\( \geq 75\% \)) recovery of fluorescence in the bleached area (5 \( \times \) 5 \( \mu \)m) within 25 s for both full-length IP\(_3\)R and each of its ER-targeted fragments. Furthermore, experiments with TMD5 and TMD6 suggest that the confocal images of misfolded and ER-targeted proteins are recognizably different. These single TMD constructs expressed more efficiently than others (necessitating a reduction in the amount of DNA used for the transfections shown in Fig. 6), but in the initial experiments, many cells expressed large amounts of aggregated protein, and the distribution of the fluorescence was obviously different from that of other constructs (not shown).

IP\(_3\)Rs are thought to assemble as a dimer of dimers, and the major determinants of assembly are TMD5 and TMD6 together with \(-25\) residues lying \(-35\) residues downstream of TMD6 (14, 15). The cytosolic determinant (residues 2629–2654 in IP\(_3\)R1; Ref. 15) is present only in full-length IP\(_3\)R and the TMR5–6C and TMR1–6C fragments, but not in the shorter fragments (Fig. 1). We predict, therefore, that fragments of the IP\(_3\)R would form dimers and tetramers only if they include TMD5 and TMD6 and only if the fragments fold properly. Covalent cross-linking of IP\(_3\)R fragments expressed in COS-7 cells confirms this prediction. Full-length IP\(_3\)R can be cross-linked into tetramers (data not shown), whereas YFP cannot: only tiny amounts of dimeric YFP were observed (Fig. 8) (and as also noted by others, see Ref. 71). Most importantly, fragments that include TMD5 and TMD6 can be cross-linked into dimers and tetramers of the expected sizes, and the presence of the C-terminal improves the cross-linking, but fragments that include only TMD1–4 fail to cross-link (Fig. 8). These results, which are entirely consistent with established determinants of IP\(_3\)R oligomerization (10, 14, 15), provide persuasive evidence that the IP\(_3\)R fragments used to define the determinants of targeting are correctly folded.

TMD5 and TMD6 alone are targeted to ER, but they pass to the plasma membrane when attached to ICAM (Fig. 6, C and D), suggesting that neither fragment was mis-folded. The post-translational targeting of TMD1 and TMD2 to mitochondria (Fig. 7) would also seem unlikely to occur for a mis-folded protein.

Another possibility is that fragments of the IP\(_3\)R are targeted to the ER not by their own intrinsic targeting sequences but by assembling with native full-length IP\(_3\)R, but this is not the case. Others have shown that, in COS-7 cells, native and ex-

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A. K. T. Parker, F. V. Gergely, C. W. Taylor, and D. Brough, unpublished observations.
pressed IP3R do not co-assemble (72); we failed to detect cross-linking of fragments with full-length IP3R (Fig. 8), and fragments that lack TMD5 and TMD6 do not form oligomers (Fig. 8) but are, nevertheless, targeted to the ER (Figs. 3, 4, and 6). ER-targeting information must, therefore, reside within the TMDs.

DISCUSSION

Redundant Signals Retain IP3R in the ER—Expression of IP3R in the ER requires that the nascent peptide is co-translationally targeted to ER by SRP and the Sec61p complex (69), and that the fully assembled protein is then retained within ER membranes, either because it is prevented from leaving or by retrieval from later compartments.

Any pair of TMD from IP3R1 targets the nascent peptide to the ER (Fig. 3). Either TMD5 or TMD6 alone is also sufficient, but neither TMD1 nor TMD2 is capable of independently targeting the nascent chain to the ER (Fig. 6); these proteins instead accumulate in mitochondria (Fig. 7). TMD are important in targeting proteins to the outer mitochondrial membrane, but it is not clear how these TMD evade SRP-mediated targeting to the ER. Analysis of Tom20, which has a single TMD and is expressed in the outer mitochondrial membrane, suggests that less hydrophobic TMD and the presence of basic residues immediately downstream allow the TMD to evade recognition by SRP and instead to be targeted to mitochondria (68). It is noteworthy that for IP3R, TMD1 and TMD2 are less hydrophobic than TMD5 and TMD6 (Fig. 9A), and (at least for IP3R1) only TMD1 and TMD2 have the flanking, positively charged residues that favor mitochondrial targeting. We suggest that full-length IP3R1 is targeted to the ER only after translation of both TMD1 and TMD2, which are together recognized by SRP. The remaining TMD would then be co-translationally inserted into the ER membrane.

After Co-translational Assembly in the ER, What Keeps IP3R There?—Any pair of TMD from IP3R1 is sufficient to mediate ER retention (Figs. 3–5); TMD5 and TMD6 alone provide weaker retention signals that allow retention of IP3R, but not of ICAM (Fig. 6). We cannot exclude the presence of additional signals lying outside the TMD, but it is clear that several redundant signals retain IP3R in the ER. By contrast, an analysis based on the ability of fragments from RyR1 to cause retention of ICAM in the ER concluded that the TMD equivalent to TMD6 of IP3R was responsible for ER retention (58). What are the TMD signals that allow retention of IP3R within ER, and how might such redundant signaling be overcome to allow targeting of IP3R to other membranes? Retention may depend upon recognition of specific residues within the TMD or their physical properties (see beginning of text), but there are no obvious similarities in either the pairs of effective TMD or between TMD5 and TMD6: they differ in length, hydrophobic-
ity, and sequence (Fig. 9). Helical wheel projections of the penultimate TMD suggest the presence of a polar face in IP$_3$R (TMD5) but not in RyR (Fig. 9B); this feature has been suggested to mediate retention of other ER membrane proteins (50, 51). The last TMD of RyR1 and IP$_3$R are very similar (TMD6) but not in RyR (Fig. 9); this feature has been suggested to mediate mitochondrial targeting, whereas more hydrophobic TMD (2.21–3.23) favor ER targeting (68). Polar residues are in bold. B, helical wheel projections of the penultimate TMD from IP$_3$R1 (underlined) and RyR1, with only polar residues shown. C, comparisons of the final TMD of IP$_3$R and RyR. Residues that are identical in all subtypes are shown in bold; polar residues are underlined.

**Fig. 9. TMD of IP$_3$R and RyR.** A, the average hydrophobicity of residues ($\pi$) within each TMD and the C-terminal flanking residues are shown for each IP$_3$R subtype. Basic flanking residues and less hydrophobic TMD (values of 1.97–2.16) favor mitochondrial targeting, whereas more hydrophobic TMD (2.21–3.23) favor ER targeting (68). Polar residues are in bold. B, helical wheel projections of the penultimate TMD from IP$_3$R1 (underlined) and RyR1, with only polar residues shown. C, comparisons of the final TMD of IP$_3$R and RyR. Residues that are identical in all subtypes are shown in bold; polar residues are underlined.

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