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In with the TRP Channels: Intracellular Functions for TRPM1 and TRPM2

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Abstract

Transient receptor potential (TRP) channels are a large family of cation channels, many of which are predominantly localized to the plasma membrane where they transduce the effects of diverse and often sensory stimuli. Two members of the TRP melastatin subfamily, TRPM1 and TRPM2, are localized in intracellular compartments and are involved in melanin synthesis and oxidative stress-induced cell death, respectively. These findings provide new insight into the location and function of TRP channels.

Changes in cytosolic calcium concentration govern a huge number of cellular processes (1). Calcium can be rapidly increased by the opening of calcium-permeable channels located on the plasma membrane, membranes of organelles that serve as intracellular calcium stores, or both (2). Whereas a multitude of calcium-permeable channels are found on the cell surface, the pallet of intracellular calcium-permeable channels is more limited. The endoplasmic reticulum is by far the best-characterized intracellular calcium store housing inositol trisphosphate-sensitive and ryanodine-sensitive calcium channels that mediate calcium release in response to the calcium-mobilizing messengers, inositol trisphosphate and cyclic adenosine diphosphate (ADP) ribose, respectively (3). Additionally, accumulating evidence indicates that various acidic organelles can also serve as calcium stores, including the so-called “acidocalcisomes” [first described in protists but likely to be ubiquitous (4)] and lysosomes, which are present in all eukaryotic cells (5). Calcium is released from lysosomes in response to the calcium-mobilizing messenger NAADP (nicotinic acid adenine dinucleotide phosphate) (6,7). However, much less is known concerning the molecular details of calcium signaling through these organelles and acidic stores in general, relative to calcium signaling mediated by the endoplasmic reticulum.

Transient receptor potential (TRP) channels are a large superfamily of distantly related cation channels involved in the transduction of a multitude of stimuli (8). TRP channels in mammals are divided into two groups: group I, comprising TRPC, TRPV, TRPM, and TRPA, and group II, including TRPP and TRPML (8). Although most TRP channels are located in and function at the plasma membrane (8), many members are also detectable in organelles of the secretory pathway, likely reflecting trafficking of presumably nonfunctional channels to the plasma membrane (9). TRPV1 (10) and TRPP2 (11) located in the endoplasmic reticulum, however, may function as intracellular calcium release channels. The TRP mucolipins (TRPMLs) are also located on intracellular membranes. These channels are named from the discovery that

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the gene encoding the founding member (TRPML1) in humans is mutated in the lysosomal storage disease mucopolipidosis IV (12). Consistent with alterations in lysosome function, TRPML1 localizes to the late endosomes and lysosomes through well-defined targeting motifs (13). The other members of this family are also localized to specific subcompartments of the endolysosomal system as well as to the plasma membrane (14). Although the channel properties of TRPMLs are the subject of controversy, several studies suggest that TRP mucolipins can function as calcium-permeable channels (14). This has led to the proposal that aberrant lysosomal release of calcium through TRPML1 underlies the derangements in trafficking associated with mucopolipidosis IV (15).

Two studies provide new evidence that members of the TRP melastatin (TRPM) family may also function as intracellular TRP channels (16,17). TRPM1, the founding member of this family, was originally characterized as the product of a gene whose expression is decreased in melanomas (18) and is useful as a prognostic marker for metastasis (19). Despite progress in the characterization of other TRPM members, the biophysical properties and function of TRPM1 were not known, although evidence for a potential role in mediating synaptic transmission in rod bipolar cells of the retina has been obtained (20).

Oancea *et al.* (16) recorded outwardly rectifying nonselective cation currents in both melanocyte cell lines and human epidermal melanocytes, and these currents were reduced in amplitude when expression of the gene encoding TRPM1 was suppressed. They also identified several previously uncharacterized splice variants of TRPM1, including two variants that encode proteins with extended N termini. Overexpression of these two variants (but not shorter variants) in melanocyte cell lines resulted in the appearance of a current that was biophysically similar to the endogenous TRPM1 current. Localization studies of green fluorescent protein (GFP)-tagged versions of the various isoforms showed that all variants localized not to the plasma membrane but instead to highly dynamic intracellular tubulovesicular structures. This unexpected intracellular location is consistent with the relatively modest magnitude of the whole-cell currents recorded in cells overexpressing TRPM1. Furthermore, Oancea *et al.* found that knockdown of TRPM1 reduced the abundance of melanin pigment α —finding confirmed independently by Devi *et al.* (21). Indeed, reduced abundance of the mRNA for TRPM1 in the skin is evident in horses with Appaloosa spotting (22), a striking coloration trait characterized by white patches of coat.

Melanin is synthesized within lysosome-related organelles known as melanosomes. How TRPM1 regulates melanin synthesis was not studied, but one possibility is that TRPM-mediated ion fluxes across the melanosome itself may regulate melanosome function. Indeed, melanosomes contain large amounts of calcium (23,24). Costaining of melanocytes for TRPM1 and a melanosomal marker, however, did not reveal any substantive overlap of the two proteins, which suggests that TRPM1 probably does not localize to the organelle (16). Thus, modulation of melanosome function by TRPM1 is likely indirect. In this context, it is interesting to note altered coat pigmentation in the Varitint-Waddler mouse, which is due to a gain-of-function mutation in the endolysosomal TRPML channel TRPML3 (25–28). Additionally, coding variants of TPC2, a member of a family of endolysosomal two-pore calcium channels (29, 30), have also been correlated with hair pigmentation in humans (31). Taken together, these data suggest a possible conserved role for intracellular ion channels in the control of pigmentation (Fig. 1).

A second study by Lange *et al.* (17) provides insight into the location and function of isoform 2 of the TRPM family. TRPM2 is an unusual protein in that it is a “chanzyme,” possessing both ion channel and enzymatic activity as an adenosine diphosphoribose (ADPR) hydrolase (32). Electrophysiological analysis clearly indicates that TRPM2 can function as a plasma membrane channel. It is a nonselective cation channel and thus permeable to calcium, and it

is activated and regulated by various factors, including cytosolic ADP-ribose (32) and oxidants such as hydrogen peroxide (33). With HEK293 cells heterologously expressing TRPM2, Lange *et al.* showed that intracellular delivery of ADP-ribose increased cytosolic calcium concentrations even when calcium was omitted from the extracellular medium. Thus, ADP-ribose evokes calcium release from intracellular calcium stores because calcium influx through plasma membrane TRPM2 channels would be absent under these conditions. Similar results were obtained with INS-1 b cells (a pancreatic b cell line) and primary mouse pancreatic b cells expressing endogenous TRPM2.

Moreover, Lange *et al.* found that calcium signals mediated by ADP-ribose were reduced in both cell types upon silencing of TRPM2 through two different methods (small interfering RNA and the use of cells from a TRPM2-knockout mouse). Endogenous TRPM2 in INS-1 b cells localized to lysosomes, which suggests that TRPM2 mediates calcium release from these organelles. In support, ADP-ribose-mediated calcium release was blocked by the vacuolar proton pump inhibitor bafilomycin A₁, which likely depletes acidic calcium stores of calcium. In contrast, blocking inositol trisphosphate receptors with heparin had little effect, although blockade of ryanodine receptors with ryanodine did inhibit calcium signaling in response to ADP-ribose. The latter result might be indicative of a possible functional interaction between lysosomal TRPM2 channels and endoplasmic reticulum-resident ryanodine receptors in a manner akin to that observed with NAADP receptors and endoplasmic reticulum calcium-permeable channels (34,35). H₂O₂-mediated cell death was reduced in cells lacking TRPM2 when experiments were performed in either the presence or absence of extracellular calcium (17), which suggests that that TRPM2 channels located at the plasma membrane and lysosomes contribute to cell death in response to oxidative stress (Fig. 1).

TRPM2 thus joins TRPMLs and the two-pore channels as lysosomal calcium release channels. It is well established that the calcium-mobilizing messenger NAADP mobilizes lysosomal or lysosome-like calcium stores in various cells, and this process is likely mediated through the two-pore channels (29,30). Given that both TRPM2 (36) and TRPML1 (37) are sensitized or activated by NAADP, it is possible that NAADP coordinates the activity of multiple intracellular calcium release channels (Fig. 1).

The studies by Oancea *et al.* (16) and Lange *et al.* (17) advance our understanding of the intracellular portfolio and function of TRP channels. Inevitably, such findings raise many more questions. For TRPM1, what is its exact intracellular location and how does it regulate melanin content? Also, the fact that only the longer TRPM1 variants are active—and then only in cell lines of melanocyte lineage [but see (38)]—suggests that cell type-specific components are required for activity, presumably through interactions with the N terminus. What are these factors? Their identification may shed light on how these channels are regulated. For TRPM2, although a pathophysiological role for this channel in oxidative stress-induced cell death has been reported, does ADP-ribose-mediated lysosomal calcium release contribute to physiological (agonist-evoked) calcium release events, particularly those shown previously to depend on NAADP? The future is bright (or perhaps dark, in the case of TRPM1) for this remarkably diverse family of ion channels.

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Fig. 1.

Intracellular functions of TRPM1 and TRPM2. TRPM1 is a calcium-permeable channel localized to vesicular structures (of unknown identity) that influence melanin synthesis in melanosomes, possibly in concert with lysosomally targeted two-pore channels (TPCs) and TRP mucolipins (TRPMLs). TRPM2 is a calcium-permeable channel localized to lysosomes (as well as the plasma membrane, not shown). It is activated by cytosolic ADP-ribose and involved in H₂O₂-mediated cell death. TPCs, TRPML, and TRPM2 may all be activated by NAADP. Bafilomycin A₁ (Baf) inhibits the vacuolar H⁺-adenosine triphosphatase, preventing H⁺ uptake into the lysosome, which in turn likely prevents Ca²⁺ uptake and subsequent Ca²⁺ release through lysosomal calcium channels.