Since adenosine 5’-diphosphoribose (ADPR) has been discovered (1) as stimulus of the human apoptosis-related ion channel transient receptor potential melastatin 2 (TRPM2), the compound has undergone a rapid ascent from being considered a mere spin-off of the cellular energy metabolism to a generally acknowledged important intracellular second messenger. In clear distinction to post-translational modifications of proteins in the process of ADP-ribosylation [e.g., reviewed in (2)], ADPR activates the Ca²⁺-permeable cation channel TRPM2 in the manner of a receptor agonist. This activation does not involve a covalent modification but constitutes a classical interaction between ADPR as a ligand to a specific binding site. The ADPR binding pocket of TRPM2 is special in so far in that it represents an intrinsic protein domain which is homologous to the human Nudix hydrolase NUDT9, a mitochondrial/cytosolic enzyme that specifically binds and hydrolyzes ADPR (1). Therefore, TRPM2 may be considered a member of the exclusive club of chanzymes. This activation does not involve a covalent modification but constitutes a classical interaction between ADPR as a ligand to a specific binding site.

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On the basis of these findings, further questions go into two directions. One is how binding of ADPR to the NUDT9 domain results in gating and which parts of the channel protein participate in which manner. The other is about the detailed mechanisms of binding. What are the structural requirements on the ligand and on the protein for binding and what decides whether binding has agonistic or antagonistic effects? It is for the latter question that Fliegert et al. provide remarkable progress with their strategy combining chemical modifications of the ligand and site-directed mutagenesis of the putative ADPR binding pocket.

As an indispensable starting point for further detailed structure-function analysis of the NUDT9H domain of TRPM2, the crystal structure of the human NUDT9 enzyme has been provided at 1.8 Å resolution (9). Based on that, predictions on structural requirements of ADPR binding to the NUDT9H domain of TRPM2 have been proposed and experimentally validated (10,11). This approach is not free from possible pitfalls, however, because NUDT9H domain and NUDT9 enzyme share only 39% of their sequences and there exist some important differences between NUDT9 and TRPM2 in terms of substrate recognition and ligand binding, most notable for O-acetyl-ADPR (9,12). As even more critical point, reservation must be kept in mind, that principally apply to comparisons of an independent soluble protein with a domain structurally and functionally integrated within an ion channel, as well as to comparisons between an enzyme and a binding domain without catalytic activity. Clearly, data from cryo-electron
microscopy or crystallography of TRPM2, rather than only of NUDT9, are highly desirable, but for the time being approaches like that of Fliegert et al. highly valuable and promising for the further understanding and exploiting of how ligand-binding to TRPM2 takes place. The particular strength of Fliegert et al.’s work is that it tests structural predictions with novel ADPR analogues and thereby not only creates insight into interactions of ligands with their binding sites but also provides new pharmacological tools—and ideas how further of those tools may be designed by expert chemists.

Already previous studies on modified ADPR analogues (13) have revealed that ADP alone had no significant effect at TRPM2 and that especially the terminal ribose moiety of ADPR is crucial for substrate recognition. Accordingly, Fliegert et al. demonstrate in whole-cell patch-clamp experiments that four different ADPR analogues lack the ability to activate human TRPM2 because of modifications of the terminal ribose. In an important extension of previous work, ADPR modified by hydrophobic substitutions at the beta-phosphate of ADP, either by a beta-methyl group or by a beta-tetrahydrofuran-2-yl-methyl (THF) group, proved to be significantly antagonistic to ADPR-dependent channel activation. The antagonistic efficacy was clearly enhanced by increasing shape similarity to the terminal ribose. Specifically it increased from beta-methyl-ADP (low) to THF-ADP (moderate) and even more to alpha-1''-O-methyl-ADPR (high). Interestingly, the anemic form beta-1''-O-methyl-ADPR showed the weakest antagonistic effect, strongly suggesting that the C1''-hydroxyl group of the terminal ribose and its proper configuration play a pivotal role in ADPR-dependent activation of TRPM2.

In a next step Fliegert et al. identified, in the NUDT9H domain of TRPM2, some potential molecular interaction partners of the terminal ribose by site-directed mutagenesis guided by homology modelling/docking. As correctly anticipated by the authors, the high flexibility of ADPR within the putative binding pocket of the channel with respect to side chain orientation hampers reliable predictions about specific interaction partners within the TRPM2 structure. This may explain why another study (10) has come to different conclusions on the potential interaction partners, although an apparently similar homology modelling approach was used. Fliegert et al. focus on five amino acid residues (T1347, Y1349, L1381, R1433, Y1485) as potential interaction partners of the terminal ribose. When two of them were individually subjected to relatively conservative amino acid substitutions (T1347V, Y1349F), functional consequences (although moderate) were confirmed in calcium imaging experiments.

A third point mutation (R1433M) induced the most pronounced functional effect (59% reduction) on TRPM2 activation. Here, however, the interpretation may be ambiguous because exactly this channel variant seems to show a reduced expression level in comparison to all other variants tested. In the simultaneous study by Yu et al. [2017], the same critical residue R1433 was either replaced by alanine, glycine, leucine, glutamine or lysine. The ADPR-stimulated whole-cell currents of these TRPM2 variants (R1433A, R1433G, R1433L, R1433Q and R1433K) were abolished (with the exception of R1433G), while the surface expression of, e.g., R1433A was indistinguishable from that of wild-type TRPM2.

As a personal comment from a lab that uses both approaches for functional studies on TRPM2, patch-clamp and calcium imaging, it may be added that the ADPR sensitivity of TRPM2 is best evaluated with whole-cell current recordings in the presence of a rigorously controlled intracellular Ca\(^{2+}\)-concentration. This method keeps the activity-boosting effects of Ca\(^{2+}\) under control, although Ca\(^{2+}\) is a co-agonist to ADPR and enters the cell through open TRPM2 channels, thereby creating a positive feedback on the channel activation (14,15).

In summary, while searching for the critical interactions between ADPR and the NUDT9H domain of the human TRPM2 channel Fliegert et al. provide convincing evidence that the terminal ribose moiety and especially the conformation of its C1''-hydroxyl group play pivotal roles for the proper binding of ADPR. Together with Yu et al. [2017], they have identified the most probable interaction partners of ADPR within the NUDT9H domain. For further studies on ADPR degradation by the NUDT9 enzyme the next logical step would be the elucidation of the catalytic mechanism that hydrolyzes ADPR upon binding. Such studies benefit from the fact that there are many Nudix-pyrophosphohydrolases widely distributed in all kingdoms of life showing similar substrate specificities (16-18). In the case of ADPR as channel activator, however, TRPM2 is the unique target. In this situation the comparative analysis of species variants might be a promising tool to elucidate the complex structural-function relationship of TRPM2. The recent discovery of a TRPM2-like channel from the sea anemone Nematostella
vectensis could be a first step in this direction (19). Although ADPR seems to interact with an additional site outside of the NUDT9H domain of the sea anemone channel (7), the basic mechanism of the subsequent gating process might well be similar.

Last but not least, the present findings by Fliegert et al. may have impact not only on the understanding of basic mechanisms of TRPM2 channel activation but moreover provide promising pharmacological and even therapeutic prospects. TRPM2 is involved in cell death (which may be apoptosis or necrosis) as well as in other conditions of medical interest, notably granulocytic inflammation. Potential inhibitors that use the principle of pore blocking can hardly be specific for TRPM2, or any other individual member of the TRP family. On the other hand, the unique activation mechanism of TRPM2 involving binding of ADPR to the NUDT9H domain offers the opportunity for highly specific pharmacological interactions. The exact information on binding requirements given by Fliegert et al. in their present paper may be of invaluable help for a rationale design of drugs targeting TRPM2 and aiming at modulation of the beneficial as well as the detrimental biological functions of this channel.

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Footnote

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