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EDITORIAL

# Orai channels – New insights, new ideas

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The basic mechanism of agonist-activated $Ca^{2+}$ entry in non-excitable cells was first defined by Jim Putney some 25 years ago (Putney, 1986). This mechanism of 'capacitative', or store-operated, $Ca^{2+}$ entry described how such entry was strictly a consequence of the depletion of intracellular $Ca^{2+}$ stores, typically those formed by the endoplasmic reticulum (ER). During agonist action such depletion would normally result from the generation of $InsP_3$, and the resulting release of ER $Ca^{2+}$, but the essential dependence on store depletion *per se* was demonstrated by the fact that the same entry could be induced by any means that resulted in a corresponding decline in the concentration of free $Ca^{2+}$ in the ER, including inhibition of SERCA pumps, or increasing $Ca^{2+}$ buffer levels in the ER. Subsequently, a highly $Ca^{2+}$-selective conductance representing such an entry pathway was identified, initially in mast cells and T lymphocytes, and its essential biophysical properties described and defined (Hoth & Penner, 1992; Zweifach & Lewis, 1993). This was the CRAC channel ($Ca^{2+}$ release-activated $Ca^{2+}$ channel). However, despite extensive analysis of the properties and behaviour of these channels, their molecular identity and the actual processes that link the depletion of intracellular $Ca^{2+}$ stores with activation of the channels remained unknown for almost 15 years. All of this changed dramatically with a series of papers that appeared in 2005 and 2006. First, the protein STIM1 was identified as the molecule linking the detection of $Ca^{2+}$ store depletion with the subsequent activation of the CRAC channels (Liou *et al.* 2005; Roos *et al.* 2005). Then, a year later, a novel family of proteins named Orai proteins were shown to form the channels themselves (Feske *et al.* 2006; Vig *et al.* 2006; Zhang *et al.* 2006). The resulting surge of new findings that resulted from this identification of the molecular basis of store-operated $Ca^{2+}$ entry was nicely summarized in a symposium organized by Anant Parekh and sponsored by *The Journal of Physiology*, which was held at the 52nd Annual Biophysical Society meeting at Long Beach, California in 2008.

So, why was *The Journal of Physiology* sponsoring another symposium entitled 'Orai channels – New insights, new ideas' just four years later at the 2012 Biophysical Society meeting in San Diego? Surely, once the molecular basis of this mode of $Ca^{2+}$ entry had been identified and characterized, all that was left to do was essentially a matter of simply 'dotting the i's, and crossing the t's'. . . . . Wrong!! In the four years since the Long Beach symposium, the field of Orai channels has expanded – perhaps 'exploded' is a more appropriate term – to reveal their multiple activities in diverse cell types, their specific features and behaviour, their interaction with a variety of other proteins, and their potential ability to assemble into distinct channel types displaying entirely unique properties and functions. It was these new, and undoubtedly still expanding, range of activities, properties, and roles that was the focus of the San Diego symposium, co-chaired by myself and Jim Putney.

Reflecting the sense that the discovery of the Orai channel proteins has marked the beginning of an entirely new era in the regulation and modulation of $Ca^{2+}$ entry, the speakers were deliberately chosen from some of the younger investigators in this area – representing the future of what is surely to be a critically important focus of research for many years to come. Perhaps not surprisingly, the first three speakers were actually the first three authors on the ground-breaking 2006 paper (Feske *et al.* 2006) identifying Orai proteins as the essential components of CRAC channels. Stefan Feske (New York University, New York) began the meeting by presenting a comprehensive and authoritative guide to the multiplicity of ways in which Orai channels are involved in different immunological responses (see Shaw & Feske, 2012). Of course, the key role that Orai channels play in immune responses was critical in their initial identification but subsequent studies have considerably expanded on this role. In particular, the complex and critical roles of Orai and STIM proteins in determining the balance between the activation of a positive, or productive, immune response relative to the initiation of an undesirable autoimmune response were detailed. Yousang Gwack (University of California, Los Angeles) then discussed the variety of different proteins (e.g. calmodulin, CRACR2A, Golli-BG21, the SPCA2 $Ca^{2+}$-ATPase, and junctate) that recent studies have shown to interact with Orai proteins and/or their regulator STIM1 to modulate or fine-tune the overall activity of these channels (see Srikanth & Gwack, 2012). Evidence suggests that both positive and negative regulators are involved, and that their effects can result from a diverse range of specific actions, including STIM1 translocation to the plasma membrane, Orai–STIM1 interactions, and actual Orai channel behaviour. It was also noted that, because many of these effects are subtle, it will be important to examine their possible contribution to long-term effects, such as gene transcription, etc. Next, Murali Prakriya (Northwestern University, Chicago) discussed the recent findings related to the molecular basis of the key biophysical properties of the Orai channels, particularly their extraordinarily high selectivity for $Ca^{2+}$ ions, and their very low single channel conductance (see McNally & Prakriya, 2012). Especially intriguing was his recent finding that, in stark contrast to all other known $Ca^{2+}$ channels, the high selectivity for $Ca^{2+}$ ions shown by Orai channels is not exclusively an intrinsic property of the channel itself, but is profoundly modulated by interactions of the channel with its regulator STIM1. Barbara Niemeyer (Saarland University, Homburg, Germany) then presented her findings on the effects of reactive oxygen species (ROS) on the activity of Orai channels, particularly in the context of the responses of regulatory T cells under inflammatory conditions, and skin melanocytes under exposure to UV (see Bogeski *et al.* 2012). These effects reflect actions on certain specific reactive cysteine residues in Orai proteins and various STIM variants. Particularly interesting were the recent studies from her group that showed that, because Orai3 lacks a specific reactive cysteine that is present in Orai1, these cells are able to modulate the impact of peroxide-mediated generation of ROS by changes in the relative expression of these two Orai species. Finally, Mohamed Trebak (Albany

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Medical College, Albany) demonstrated that the functional importance of Orai channels is not limited to non-excitable cells by discussing the evidence of significant roles played by these channels in various muscle cell types (see Trebak, 2012). In particular he discussed new evidence demonstrating separate store-operated and store-*independent* Orai channels in vascular smooth muscle cells, and their selective activation by distinct agonists. Critically, it seems that the latter may play a key role in the formation of expanded intima that result from damage to the vessel wall, with obvious implications for the development, and potential remediation, of vascular disease.

This issue of *The Journal of Physiology* presents a series of short reviews by each of the above speakers at the symposium. Together, they serve as examples that illustrate the diverse range of activities carried out by Orai channels in different cell types, and the variety of ways these activities can be initiated and regulated. Finally, one point that is perhaps worth emphasizing is that, when these proteins were first identified in 2006, it was noted that they were entirely unique, displaying no similarities with any other known channel proteins. Given this, perhaps we should not be surprised if these channels do not always obey the 'existing rules' established for other channels, even other $Ca^{2+}$-selective channels, and should always keep an open mind about what these channels can do, and what factors impact their activity, properties and functions.

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