to maintain sensitivity to calcium stores and (ii) TRPC1 channels were activated by other pathways. In cells overexpressing STIM2 proteins were activated by other pathways. In cells overexpressing STIM2 proteins, ORAI does not contribute to store-operated Ca2+ entry.

Depletion of intracellular calcium stores activates store-operated channels. This process induces numerous intracellular signaling events. The most studied store-operated channels are CRAC channels. Other channels are believed to consist of TRPC and Orai proteins. However, their role in molecular composition of endogenous store-operated non-CRAC channels remains obscure. One of the main questions to ask is how TRPC channels are activated after store depletion. Most studies have used whole-cell patch clamp or calcium imaging techniques. To discriminate different types of store-operated channels, we used single-channel patch-clamp recordings in HEK293 cells. We showed that in experiments with dominant-negative mutant Orai1 E106Q endogenous TRPC1-composed channels were not sensitive to store depletion, but they were activated by other pathways. In cells expressing STIM2, proteins endogenous TRPC1 channels were activated with a delay (similarly to Orai channels activated by STIM2). In summary, we propose that (i) ORAI does not serve as a pore forming subunit of endogenous TRPC1 channels, but it is necessary to maintain sensitivity to calcium stores and (ii) TRPC1 channels are activated downstream to ORAI channels after store depletion. This study was supported by the Russian Scientific Foundation, Project 14-14-00720 (to E. K., D.K. and A.S.); and the Russian Foundation for Basic Research Project 16-04-01792 (A.S. and L.G.).

2384-Plat
The Two-Pore Domain K+ Channel TALK-1 Provides a Countercurrent That Facilitates Endoplasmic Reticulum Ca2+ Leak
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The two-pore domain K+ (K2P) channel TALK-1 modulates insulin secretion by limiting β-cell electrical excitability and cytosolic Ca2+ (Ca2++, ) influx.

2383-Plat
Role of Orai Proteins in Activation of Endogenous TRPC1-Composed Channels
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Depletion of intracellular calcium stores activates store-operated channels. This process induces numerous intracellular signaling events. The most studied store-operated channels are CRAC channels. Other channels are believed to consist of TRPC and Orai proteins. However, their role in molecular composition of endogenous store-operated non-CRAC channels remains obscure. One of the main questions to ask is how TRPC channels are activated after store depletion. Most studies have used whole-cell patch clamp or calcium imaging techniques. To discriminate different types of store-operated channels, we used single-channel patch-clamp recordings in HEK293 cells. We showed that in experiments with dominant-negative mutant Orai1 E106Q endogenous TRPC1-composed channels were not sensitive to store depletion, but they were activated by other pathways. In cells expressing STIM2, proteins endogenous TRPC1 channels were activated with a delay (similarly to Orai channels activated by STIM2). In summary, we propose that (i) ORAI does not serve as a pore forming subunit of endogenous TRPC1 channels, but it is necessary to maintain sensitivity to calcium stores and (ii) TRPC1 channels are activated downstream to ORAI channels after store depletion. This study was supported by the Russian Scientific Foundation, Project 14-14-00720 (to E. K., D.K. and A.S.); and the Russian Foundation for Basic Research Project 16-04-01792 (A.S. and L.G.).

2381-Plat
Ca2+ Signals Originate from Immobile IP3 Receptors at ER-P MJ Junctions Nagendra Babu Thillaiappan, Alap P. Chavda, Stephen C. Tovey, David L. Prole, Colin W. Taylor.
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Insoluble 1,4,5-triphosphate receptors (IP3Rs) are ubiquitous intracellular Ca2+ channels, which upon activation by IP3 and Ca2+, release Ca2+ from the endoplasmic reticulum (ER). Regulation of IP3Rs by Ca2+ spatiotemporal organization of these Ca2+ signals showed that Ca2+ signals, whether evoked by photolysis of caged IP3, or activation of endogenous receptors that stimulate IP3 formation, originate from immobile IP3Rs at ER-plasma membrane (PM) junctions. These Ca2+ release sites closely approach the ER-PM junctions where stromal-interaction molecule (STIM), the ER Ca2+-sensor that stimulates store-operated Ca2+ entry (SOCE), accumulated after depletion of ER Ca2+ stores. Our results show that IP3-evoked Ca2+ signals are initiated by immobile IP3R clusters tethered near the ER-PM junctions at which SOCE occurs. We suggest that this organization may both optimize delivery of IP3 to IP3Rs and allow effective regulation of SOCE by local depletion of Ca2+ stores.