Intracellular free Ca^{2+} regulates essential keratinocyte processes including synthesis of proteins characteristic of early differentiation, crosslinking and processing of essential structural proteins and the secretion of lipids required for the epidermal permeability barrier. In this issue of the JID, Fatherazi et al (p. 120) characterize a "Calcium-Release Activated Ca^{2+} Current", or ICRAC, in human gingival keratinocytes. ICRAC is a Ca^{2+} influx pathway that is activated by the emptying of keratinocyte Ca^{2+} stores. In keratinocytes, ICRAC then triggers other, long-lasting currents that amplify Ca^{2+} influx. These experiments thus identify the current likely responsible for capacitive, or store-operated, Ca^{2+} influx in cultured human keratinocytes, a major signaling pathway in other epithelial cells. The article also furnishes a cautionary tale of how functional and molecular identification of channels may not always coincide.

Ca^{2+} signaling is ubiquitous in eukaryotic cells. There is a substantial and tightly controlled difference between intracellular free Ca^{2+} (approximately 100 nM) and extracellular Ca^{2+} concentrations (1–3 mM). Therefore, Ca^{2+} can enter the cell driven solely along this concentration gradient, through proteins embedded in the plasma membrane known as ion channels. These channels conduct ions with varying selectivity, with entry of ions that pass through them regulated by different agents, such as changes in plasma membrane voltage, Ca^{2+} store emptying, or release of second messengers such as intracellular Ca^{2+}. For years, understanding of Ca^{2+} influx pathways in nonexcitable cells, such as keratinocytes, lagged far behind that in excitable cells, such as nerve or muscle. In 1986, James Putney (1986) first advanced the "capacitive Ca^{2+} entry" model, which proposed that Ca^{2+} entered the cell in response to emptying of intracellular Ca^{2+} stores. In 1992, Hoth and Penner (1992) identified a specific current in mast cells that was activated after intracellular Ca^{2+} stores were emptied.

Increased extracellular Ca^{2+}, the stimulus for the "Ca^{2+} switch", binds to a plasma membrane calcium receptor (CaR), producing the second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_{3}) (Fig. 1). IP_{3} induces Ca^{2+} release by binding to IP_{3}-specific receptors in the membranes of intracellular organelle Ca^{2+} stores, where free Ca^{2+} concentrations measure approximately 300 μM, again much higher than occur in the cytoplasm. Traditionally IP_{3}-responsive store Ca^{2+} has been thought to reside only in the endoplasmic reticulum. However, recent reports indicate that the Golgi also may store Ca^{2+} that may be important in keratinocyte Ca^{2+} signaling (Behne et al., in press). Analogous to plasma membrane ion channels, IP_{3} receptors function as second messenger-gated ion channels that allow Ca^{2+} to flow from the organelle interior to the cytoplasm. Intracellular Ca^{2+} release is amplified by Ca^{2+} influx from the extracellular compartment, passing through ion channels located in the plasma membrane. Fatherazi et al (2003) have experimentally reproduced the last step of this cascade, by pharmacologically emptying intracellular Ca^{2+} stores with IP_{3}, Ca^{2+} chelators such as BAPTA, or thapsigargin, which empties Ca^{2+} stores sequestered by the Ca^{2+}-ATPase ATP2A2. They find that emptying organelle Ca^{2+} stores produces a transient, Ca^{2+}-selective current that possesses many of the biophysical hallmarks of other ICRAC. In addition, large, sustained currents carrying Ca^{2+} or Na^{+} are activated subsequent to the ICRAC. Together, these currents carry a sustained Ca^{2+} influx into keratinocytes, and thus may signal early Ca^{2+}-induced differentiation.

Unlike voltage-gated Ca^{2+} channels, the molecular identity of most store-operated Ca^{2+} influx channels still has not been determined. In recent years, intense interest has focused on the three major classes of human transient receptor potential (TRP) channels: TRPC (canonical), TRPV (vanilloid) and TRPM (melastatin). In general, TRPC channels are Ca^{2+}-selective or cation-selective Ca^{2+} permeable channels that are activated by store depletion, DAG (TRPC3 or TRPC6), or IP_{3} (TRPC3). TRPV channels, in contrast, are variably selective for Ca^{2+}, activated by osmolarity, capacsin, H^{+}, or temperature, and they include the Ca^{2+}-selective epithelial Ca^{2+} channels ECA1 and 2 (renamed TRPV5 and 6). A heat-sensitive TRPV3 channel recently has been identified in keratinocytes (Pieper et al., 2002), but its characteristics do not match those of the channel studied in the current article. TRPC1, TRPC4 and TRPV6 each have been proposed to underlie ICRAC currents (see Zitt et al., 2002 for review).

Earlier keratinocyte studies using Ca^{2+}-sensitive dyes had demonstrated that significant Ca^{2+} entry follows emptying of intracellular stores (Csernoch et al., 2000). The current report, which identifies the keratinocyte ICRAC, represents a significant advance in our understanding of keratinocyte Ca^{2+} signaling because it clearly identifies a current that has, up to now, eluded precise characterization. ICRAC have been difficult to characterize because of their small conductance; thus, the current passing through an individual channel can be less than background noise in typical electrophysiological experiments. In addition, the experimental conditions that activate ICRAC can differ among cells, and the ICRAC is modified substantially by the activity of the ATP2A2 Ca^{2+}-ATPase and the amount of mitochondrial Ca^{2+} buffering (see Parekh, 2003 for review). Thus, this report represents not only the beginning of the studies that will enable us to define the specific characteristics of this channel, and to determine the contributions of the ATP2A2 and mitochondria in shaping the ICRAC. Perhaps even more exciting, these studies delineate a Ca^{2+} signaling cascade in keratinocytes that incorporates different types of channels to propagate the initial Ca^{2+} signal. In particular, ICRAC in keratinocytes is amplified by Ca^{2+} passing through different, Ca^{2+}-activated plasma membrane channels. These channels, which are activated soon after the ICRAC allow Ca^{2+} influx to persist long after the ICRAC has inactivated.

The field of channel physiology currently enjoys two powerful methods of channel identification: (a) molecular biological
Ca\textsuperscript{2+} ICRAC differs in important ways, such as current duration, from the fact that the biophysical characteristics of the keratinocyte Ca\textsuperscript{2+} ever, it is not even clear whether the ICRAC described in this report activates subsequent to the ICRAC as well. Is the Ca\textsuperscript{2+} described ICRAC’s. This confusion extends to the channels that activate the ICRAC, and possibly also the Golgi (denoted with ?) by binding to IP3 receptors (IP3R). Emptying of ER Ca\textsuperscript{2+} stores activates I\textsubscript{CRAC}, either by a direct interaction between the ER and plasma membrane or by the action of an as yet unidentified diffusible substance on the I\textsubscript{CRAC} (denoted by ?). Intracellular Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} influx through the I\textsubscript{CRAC} raise cytoplasmic (cyt) free Ca\textsuperscript{2+}. This Ca\textsuperscript{2+} signal is amplified by Ca\textsuperscript{2+} entry through the I\textsubscript{NSC}, which is activated either by Ca\textsuperscript{2+} flux through the I\textsubscript{CRAC} (denoted by ?) or directly by raised cytoplasmic Ca\textsuperscript{2+}.

Figure 1. Proposed Pathways for Ca\textsuperscript{2+} Release and Capacitive Ca\textsuperscript{2+} Influx in Keratinocytes. Raised extracellular Ca\textsuperscript{2+} is proposed to bind to a Ca\textsuperscript{2+} receptor (CaR), located in the plasma membrane and the Golgi, producing the second messenger inositol-1,4,5-trisphosphate (IP3). IP3 causes Ca\textsuperscript{2+} release from the endoplasmic reticulum (ER) and possibly also the Golgi (denoted with ?) by binding to IP3 receptors (IP3R). Emptying of ER Ca\textsuperscript{2+} stores activates I\textsubscript{CRAC}, either by a direct interaction between the ER and plasma membrane or by the action of an as yet unidentified diffusible substance on the I\textsubscript{CRAC} (denoted by ?). Intracellular Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} influx through the I\textsubscript{CRAC} raise cytoplasmic (cyt) free Ca\textsuperscript{2+}. This Ca\textsuperscript{2+} signal is amplified by Ca\textsuperscript{2+} entry through the I\textsubscript{NSC}, which is activated either by Ca\textsuperscript{2+} flux through the I\textsubscript{CRAC} (denoted by ?) or directly by raised cytoplasmic Ca\textsuperscript{2+}.

Identification of channel proteins; and (b) patch clamp identification of biophysical function. Unfortunately, either of these two powerful approaches may supply data that cannot be related to the information furnished by the companion methodology. This study illustrates these pitfalls, as the authors acknowledge. Specifically, even through several store-operated channel proteins have been identified in keratinocytes, it is not clear which protein underlies the keratinocyte I\textsubscript{CRAC}. Further complicating this issue is the fact that the biophysical characteristics of the keratinocyte I\textsubscript{CRAC} differ in important ways, such as current duration, from other reported I\textsubscript{CRAC}. These differences may be due to current modification by the ATP2A2 or mitochondria. At present, however, it is not even clear whether the I\textsubscript{CRAC} described in this report stems from the same channel(s) that underlie previously described I\textsubscript{CRAC}. This confusion extends to the channels that activate subsequent to the I\textsubscript{CRAC} as well. Is the Ca\textsuperscript{2+} -activated, Ca\textsuperscript{2+} -permeable current passing through a previously described Ca\textsuperscript{2+} activated nonselective cation channel (Mauro et al., 1995)? Is the Na\textsuperscript{+} selective current (NA\textsuperscript{+}) passing through an ENaC channel? The gold standard of channel identification involves cloning the channel, expressing it in a cell that normally does not express the channel, and determining whether the biophysical characteristics of the cloned channel match those seen in the keratinocyte. Alternative approaches are to disable or delete the candidate channel using antisense, RNAi or mice in which the channel has been ablated. We may have to await such additional experiments before the specific channels described in this report are definitively identified.

Finally, the I\textsubscript{CRAC} reported here were studied in gingival, but not in epidermal keratinocytes. Gingival keratinocytes display a divergent pattern of differentiation from epidermal keratinocytes, particularly in the later stages of differentiation. Specifically, oral keratinocytes do not cornify completely, nor do they produce lamellar bodies. Thus, extrapolation from gingival to epidermal keratinocyte signaling should be done with considerable caution. However, early differentiation in response to Ca\textsuperscript{2+} is similar in gingival and epidermal keratinocytes (reviewed in Presland and Dale, 2000). Therefore, although gingival keratinocytes differ from epidermal keratinocytes in several morphologic characteristics, the early IP3-mediated Ca\textsuperscript{2+} signaling in these two types of keratinocytes is likely to be similar.

REFERENCES


