increase reflects a growing interest in the cellular uptake of retinol, an area we are just beginning to understand. The lack of good *in vitro* models and the complexity of *in vivo* systems make it a challenging task to unravel the mechanisms of retinol uptake. Our understanding of STRA6 function was increased significantly by recent advances in cellular (Skazik *et al.*, 2014; Zhong *et al.*, 2013) and biological models (Ruiz *et al.*, 2012; Berry *et al.*, 2013). The generation of STRA6-null mice provides a cornerstone for additional biological studies. Three independent groups generated STRA6-null mice, and reported marked ocular defects in them (Ruiz *et al.*, 2012; Berry *et al.*, 2013). Thus, STRA6 is essential for retinol homeostasis in the eye. Importantly, similar ocular defects were observed in humans harboring mutations in STRA6 (Chassaing *et al.*, 2013). The reports on the STRA6-null mice argue that STRA6 is not the only pathway for retinol uptake (Ruiz *et al.*, 2012; Berry *et al.*, 2013). This sets off a search for additional transmembrane retinol transporters. In this respect, it is interesting to note that the Importin-like model of STRA6 predicts heterodimeric complexes (Figure 1), possibly with an unidentified transporter. One candidate for this protein is the recently identified RBP4 receptor-2 (RBPR2), a protein with 40% similarity to STRA6 (Alapatt *et al.*, 2013). Alternatively, RBPR2 may provide a pathway for retinol uptake which functions independently of STRA6 (Berry *et al.*, 2013). Obtaining a crystal structure of STRA6 will be a very important step in revealing its mechanism of action, but this must be complemented with studies identifying STRA6-interacting proteins.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Calcium, Orai1, and Epidermal Proliferation

Daniel D. Bikle[1] and Theodora M. Mauro[2]

Ca^{2+} influx controls essential epidermal functions, including proliferation, differentiation, cell migration, itch, and barrier homeostasis. The Orai1 ion channel allows capacitive Ca^{2+} influx after Ca^{2+} release from the endoplasmic reticulum, and it has now been shown to modulate epidermal atrophy. These findings reveal new interactions among various Ca^{2+} signaling pathways and uncover novel functions for Ca^{2+} signaling via the Orai1 channel.

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Epidermal Ca^{2+} has long been recognized as an essential signal for many epidermal functions. Beginning with early descriptions of the keratinocyte differentiation response, changes in extracellular and intracellular Ca^{2+} have been shown to direct keratinocyte proliferation, differentiation, and barrier homeostasis (reviewed in Mascia *et al.* (2012)). The marked Ca^{2+} gradient present in the epidermis, almost 4-fold higher in the stratum granulosum than in the basal layer, suggests that Ca^{2+} signaling seen in the culture dish is reflected in the *in vivo* responses of the epidermis. This report, ''Reversal of Murine Epidermal Atrophy by Topical Modulation of Calcium Signaling'', by Darbellay *et al.* (2014) reveals that Ca^{2+} flux through the plasma membrane Orai1 channel additionally controls epidermal proliferation and thickness, particularly when the epidermis atrophies in response to aging or chronic corticosteroid topical application. Related recent reports demonstrate further that the Orai1 channel also controls keratinocyte focal adhesion turnover (Vandenberghe *et al.*, 2013) and modulates early aspects of keratinocyte differentiation (Numaga-Tomita and Putney, 2013).

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Clinical Implications

- Changes in extracellular and intracellular Ca^{2+} have been shown to direct keratinocyte proliferation, differentiation, and barrier homeostasis.
- Both Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular sources are required for normal biologic responses.
- Ca^{2+} influx through the Orai1 channels enhances keratinocyte and epidermal proliferation and migration. In contrast, Ca^{2+} influx through TRPC1 and TRPC4 channels appears to direct keratinocyte differentiation.

Ca^{2+} Store Release

Keratinocytes, like many other non-excitable cells, employ Ca^{2+} signaling through a variety of pathways. Many of these pathways share common components (Figure 1). A variety of stimuli (growth factors such as EGF, ATP PAR2 receptor agonists, or raised extracellular Ca^{2+}) bind to their receptors and generate IP3, leading to Ca^{2+} release from both the endoplasmic reticulum and the Golgi. As opposed to many other mammalian cells, both of these cellular Ca^{2+} stores are important in keratinocytes, as mutations in either of the Ca^{2+} ATPases that restore these Ca^{2+} stores cause the blistering diseases Darier Disease or Hailey-Hailey Disease (reviewed in Foggia and Hovnanian (2004)). However, much less is known about Golgi Ca^{2+} signaling in keratinocytes, and this review will concentrate on the interplay between ER Ca^{2+} release, store-operated Ca^{2+} entry (SOCE) through plasma membrane ion channels, and the multiple downstream effects that are mediated by these processes. Other important signaling mediators, in particular, diacylglycerol (DAG), a protein kinase C (PKC) activator, interact with Ca^{2+} signaling to modulate keratinocyte and epidermal proliferation, differentiation, and cell-to-cell adhesion (Figure 1).

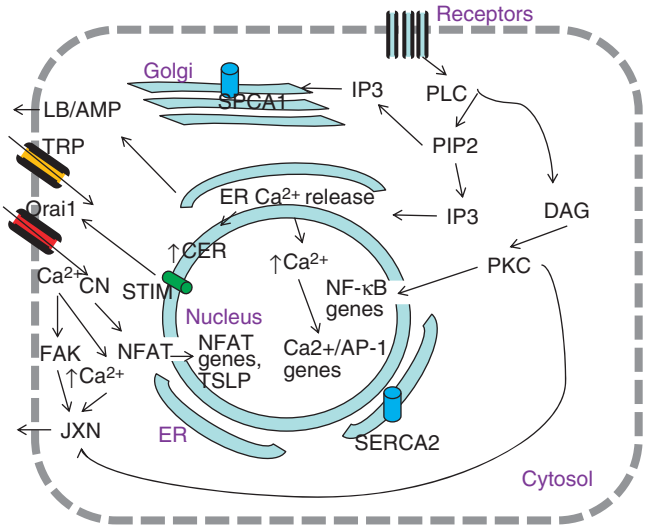

Figure 1. Agonists (e.g., EGF, ATP, Ca^{2+}, PAR2 receptor agonists) bind to their receptors and activate PLC. PLC activation, via PIP2, generates IP3, which binds to IP3 receptors and leads to ER and Golgi Ca^{2+} release. PLC also generates DAG, which, in turn activates PKC. The ER Ca^{2+} and Golgi Ca^{2+} stores are refilled by the translocation of STIM to the plasma membrane, activating the Orai1 and TRP ion channels to generate store-operated Ca^{2+} entry. Ca^{2+} ATPases SPCA1 and SERCA2 also replenish Golgi and ER Ca^{2+} stores, respectively. ER Ca^{2+} release depletes ER Ca^{2+} stores, leading immediately to lamellar body/antimicrobial peptide secretion, and also modulating cell-to-cell adhesion and migration via cytosolic Ca^{2+} and PKC or FAK activation. ER Ca^{2+} release then activates several pathways. First, Ca^{2+} entry causes nuclear translocation of NFAT via calcineurin, inducing transcription of various proteins that control differentiation and proliferation, and also TSLP (Wilson *et al.*, 2013). Next, PKC activation leads to NF-KB activation, which in turn leads to various genes that control proliferation and differentiation (reviewed in Mascia *et al.* (2012)). Ca^{2+} also modulates cell-to-cell adhesion through direct action on junctions and also through Ca^{2+} influx through Orai1 channels acting on FAK signaling pathways (Vandenberghe *et al.*, 2013). Finally, ER Ca^{2+} release generates ceramide signaling pathways, via the STAT1/3 and NF-KB signaling pathways, which in turn generate antimicrobial peptide synthesis (Park *et al.*, 2011). DAG, diacylglycerol; FAK, focal adhesion kinase; NFAT, nuclear factor of activated T cells; PKC, protein kinase C; PLC, phospholipase C; STIM, stromal interaction molecule; TSLP, thymic stromal lymphopoietin.

Both Ca^{2+} release and Ca^{2+} influx are required for normal biologic responses

ER Ca^{2+} release leads to a transient spike in cytosolic Ca^{2+}, which has rapid effects on actin reorganization and the initiation of cell-to-cell junctions. Activation of growth factor receptors such as EGFR promotes these transient spikes of calcium. Raised cytosolic Ca^{2+} also increases nuclear Ca^{2+} concentrations, which control synthesis of differentiation specific proteins such as involucrin via AP-1 binding sites (Ng *et al.*, 2000). However, this rapid cytosolic increase must be augmented by a subsequent and longer-lasting influx of Ca^{2+} through plasma membrane ion channels to effectively promote differentiation, mediated at least in part by the formation of the E-cadherin/catenin membrane complex (Bikle *et al.*, 2012). The calcium sensing receptor is instrumental in promoting these processes (Tu *et al.*, 2012). ER Ca^{2+} release also promotes epidermal permeability barrier homeostasis, as simply releasing ER Ca^{2+} by topically applying low concentrations of the irreversible SERCA2 inhibitor thapsigargin mimics lamellar body and lipid secretion, and stimulates the formation of transitional cells seen after experimental barrier perturbation (Celli *et al.*, 2011). ER Ca^{2+} release also signals antimicrobial peptide (AMP) synthesis and secretion via ceramide metabolism through the C1P/STAT1/3 and NF-kB pathways (Park *et al.*, 2011). Although extracellular Ca^{2+} seems to be required, whether and how the Orai1 channel modulates these processes is unknown. Ca^{2+} influx through the Orai1 channel, signaling via the NFAT pathway, has recently been shown to regulate thymic stromal lymphopoietin (TSLP) release from keratinocytes. TSLP then is secreted from the keratinocytes, and it subsequently activates TRPA1-positive sensory neurons to trigger itch

(Wilson *et al.*, 2013). This signaling pathway has been shown to be central to the pathogenesis of atopic dermatitis.

Different Ca^{2+} signaling processes yield different epidermal responses

The Ca^{2+} signaling processes described above display many areas of overlap, and it has not been clear how diametrically opposite results (e.g., proliferation and differentiation) could result from similar signaling pathways. However, from this and other reports, it is becoming increasingly clear that Ca^{2+} influx through the Orai1 channels appears to enhance epidermal proliferation and migration. These processes are regulated by activation of receptors such as EGFR. In contrast, Ca^{2+} influx through the TRP channels, in particular TRPC1 and TRPC4, appear to direct keratinocyte differentiation (Tu *et al.*, 2005). Recent studies show that these different outcomes may be due to the Ca^{2+} pools that are accessed, the duration of Ca^{2+} influx, ratio of stromal interaction molecule (STIM) to Orai1 proteins, relative activity of TRP versus Orai1 channels controlled by membrane depolarization, and possible direct interactions between TRP and Orai1 channels (reviewed in Saul *et al.* (2013)).

Translation to therapy?

How these findings may be translated to therapy is not yet clear. This report demonstrates that ER Ca^{2+} release and subsequent Orai1 activation, via transient SERCA2 inhibition, leads to epidermal proliferation and reversal of corticosteroid-induced epidermal atrophy. However, caution is required before attempting to treat epidermal atrophy with SERCA2 inhibitors. First, although minor SERCA2 inhibition promotes many beneficial effects, such as barrier homeostasis and normalization of epidermal atrophy, major SERCA2 inhibition is the cause of Darier Disease, a blistering skin disease caused by mutations in SERCA2 (reviewed in Foggia and Hovnanian (2004)). Second, heterozygous SERCA2 mice spontaneously develop cutaneous squamous cell carcinomas with increased expression of the oncogene K-ras (Prasad *et al.*, 2005). Thus, activating Orai1 by inhibiting SERCA2 will require more selective SERCA2 inhibitors or more selective Orai1 agonists.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Innate Immune Sensors Stimulate Inflammatory and Immunosuppressive Responses to UVB Radiation

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Almost 40 years from when it was first reported that UVB radiation exposure would modulate immune signaling, the photoimmunology field is still trying to understand the mechanisms by which UVB initiates inflammatory responses and modulates immune recognition. This commentary focuses on the ability of

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