Preventing a Perm with TRPV3

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DOI 10.1016/j.cell.2010.03.043

Epidermal growth factor receptor (EGFR) signaling is instrumental for terminal differentiation of keratinocytes, hair morphogenesis, and maintenance of the skin barrier. Cheng et al. (2010) now demonstrate that the calcium-permeable channel TRPV3 is required for these EGFR-dependent functions.

Skin is our first line of defense against infection, dehydration, chemical irritants, and ultraviolet light. Added protection from environmental assaults and thermal insulation is provided by hair, a specialized type of skin appendage. The outer layer of the skin, the epidermis, undergoes constant renewal regulated by signaling through the epidermal growth factor receptor (EGFR), which controls proliferation and terminal differentiation of keratinocytes. As a result, a deficit in EGFR signaling has multiple effects, including curling of the hair and whiskers and impairment of the skin barrier. In this issue, Cheng et al. (2010) demonstrate that a member of the transient receptor potential (TRP) superfamily of cation channels, TRPV3, is present in a molecular complex with EGFR and is required for late terminal differentiation of keratinocytes, hair morphogenesis, and skin barrier maintenance.

This discovery builds on prior work on *waved-1* (*wa1*) and *waved-2* (*wa2*) mouse mutants, known since the 1930s, which have curled whiskers and hair—resembling a perm. This phenotype has more recently been shown to result from mutations in EGFR and one of its ligands, transforming growth factor-α (TGF-α) (reviewed in Schneider et al., 2008). Once activated by TGF-α, EGFR couples directly or indirectly to several signaling pathways and effectors, such as the MAP kinase cascade, phospholipase Cγ (PLCγ), PI-3 kinase, and protein kinase C. EGFR signaling is also regulated by Ca^{2+}-mediated positive feedback. An initial rise in intracellular Ca^{2+} activates the proteases (sheddases) that release the TGF-α (Figure 1A). Thus, engagement of the ligand with EGFR leads to a further increase in Ca^{2+}, thereby liberating more TGF-α and heightening the activity of the receptor (Figure 1B). Despite the many roles for Ca^{2+} in EGFR signaling, the Ca^{2+} influx channel(s) that act in this

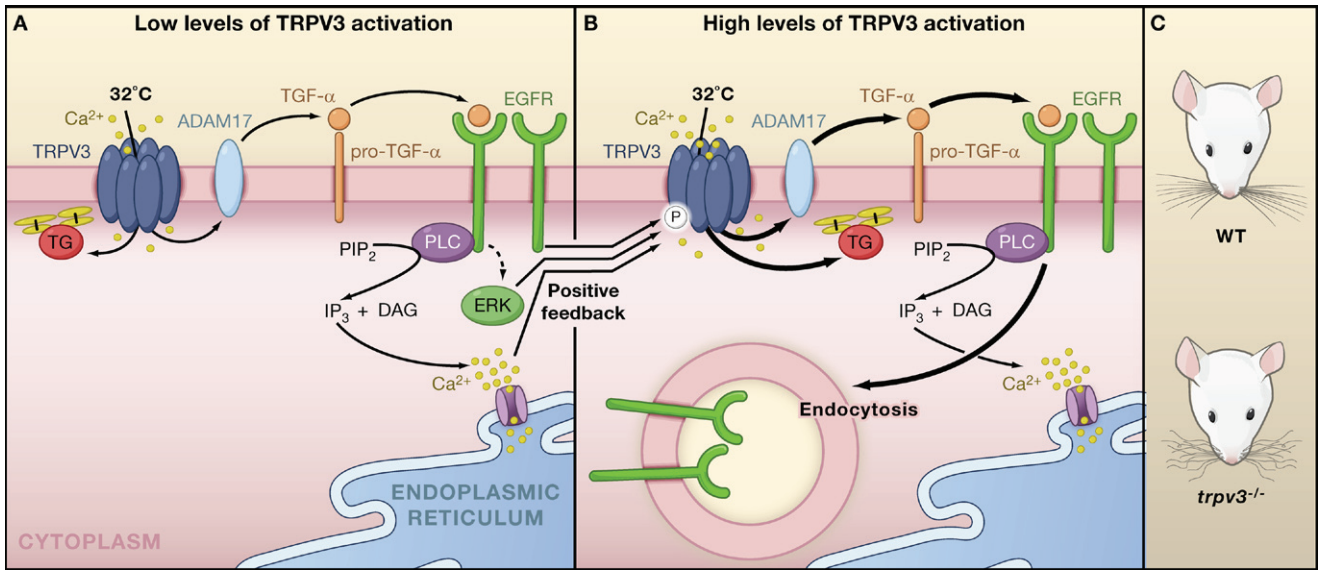

Figure 1. TRPV3 Impacts EGFR Signaling in Hair Morphogenesis
(A and B) Model for positive and negative feedback regulation of epidermal growth factor receptor (EGFR) signaling. (A) Low levels of TRPV3 activity. When TRPV3 is constitutively activated at a low level, as might occur at skin temperature, a weak influx of Ca^{2+} ions leads to low-level activity of tranglutaminase (TG) and the metalloprotease ADAM17. ADAM17 releases low levels of TGF-α, which lead to a low level of EGFR activation. Subsequent phosphorylation of TRPV3, activation of the kinase ERK, and phospholipase C (PLC), followed by inositol trisphosphate (IP_3) production and Ca^{2+} release from the endoplasmic reticulum (ER), might potentiate TRPV3 activity, as shown in (B). (B) High levels of TRPV3 activation. Strong Ca^{2+} influx leads to high levels of TG and ADAM17 activity, which releases high levels of TGF-α. Induction of high EGFR activity promotes endocytosis and degradation of a fraction of the EGFR pool.
(C) Mice deficient in *trpv3* (*trpv3*$^{-/-}$) have curled whiskers and hair, resembling a perm.

pathway in the epidermis were, until now, not known. Members of the TRP superfamily of cation channels are intriguing candidates given that most are Ca^{2+} permeable and several are expressed in skin, and there is a precedent for the activation of a TRP channel by a receptor tyrosine kinase in cells isolated from the brain (Li et al., 1999).

Cheng et al. demonstrate that the TRPV3 channel is an integral part of the EGFR signaling pathway and is required for terminal differentiation of hair, whiskers, and the skin barrier. Prior to this study, TRPV3 has been shown to be thermally activated by mild warm temperatures that are typical of the skin surface and to be expressed in keratinocytes, including follicular keratinocytes, which are needed for generation and morphogenesis of hair and whiskers. Cheng et al. produced a "floxed" allele of TRPV3, thereby allowing for either global or keratinocyte-specific knockout of the gene. All of the mutant animals exhibit curled and hooked whiskers and hair reminiscent of the *wa1* and *wa2* phenotypes (Figure 1C). The complete penetrance of this phenotype is surprising, given that hair irregularity only affects subset of the previously reported *trpv3* knockout mice (Moqrich et al., 2005). Cheng et al. also find that their knockout mice are impaired in terminal differentiation of the keratinocytes but not in proliferation of the cells in the basal layer.

Of particular importance, the authors show that both expression of TGF-α and shedding of this ligand depend on TRPV3. Consequently, the activity of the EGFR receptor is reduced in *trpv3* knockout mice. Conversely, the amount of EGFR is increased slightly, presumably due to decreased negative feedback of EGFR signaling, which is normally mediated through endocytosis and degradation of the receptor. Given that the TRPV3-dependent rise in cytosolic Ca^{2+} is needed for release of the TGF-α ectodomain by the metalloprotease ADAM17, the question arises as to how TRPV3 gets activated prior to engagement of the ligand with the EGFR. The authors put forth the intriguing concept that skin surface temperature ($\sim$32°C) induces a relatively low level of TRPV3 opening (Figure 1A), and that subsequent stimulation of the EGFR pathway augments channel activity (Figure 1B). In support of this notion, Cheng et al. find that that thermal activation of TRPV3 in cultured keratinocytes is enhanced by stimulation with TGF-α. A future direct test of this model in vivo would require knocking in a derivative of TRPV3 with one of the known mutations in the sixth transmembrane that specifically affects temperature but not chemical activation (Grandl et al., 2008).

Another question concerns the mechanism through which activation of TGF-α and EGFR enhances TRPV3 activity. The answer appears to be complex as inhibitors of either PLC or ERKs (also known as MAP kinases) reduce TGF-α potentiation of TRPV3 activity. Furthermore, TRPV3 interacts either directly or indirectly with EGFR and is tyrosine phosphorylated, which might also contribute to potentiation of TRPV3 activity. There are multiple possible modes through which PLCγ activity could couple to TRPV3. These include production of inositol trisphosphate (IP_3) and release of Ca^{2+} from the endoplasmic reticulum—a scenario reminiscent of the TrkB/TRPC3 complex in native pontine neurons, which leads to activation of TRPC3 through a mechanism dependent on IP_3 and Ca^{2+} (Li et al., 1999). It is also an open question as to how ERK activity is coupled to TRPV3. Although the nuclear functions of ERK have received the most attention, substrates for this protein kinase include cytoskeletal proteins and ion channels (Yoon and Seger, 2006). Thus, ERK might potentiate TRPV3 either via direct phosphorylation or through effects on cortical actin.

Among the salient phenotypes of the *trpv3* knockout mice is impaired skin barrier formation. As they proceed on their upward journey to the skin surface, keratinocytes ultimately lose their nucleus and other organelles and turn into flattened corneocytes that line the outermost portion of the epidermis. In *trpv3* knockout mice, the cornified envelopes are thinner than in wild-type mice. The authors provide a molecular mechanism for this deficit by showing that the activities of transglutaminases (Ca^{2+}-dependent enzymes that crosslink proteins to help establish the cell envelope) are impaired in keratinocytes lacking TRPV3.

The work by Cheng et al. represents the first link between TRPV3 and EGFR signaling. In light of these findings, the previous studies on mice expressing a constitutive active allele of TRPV3 also pointed to this connection. Surplus EGFR signaling in keratinocytes leads to hairless mice, a phenotype that is also observed in rodents with excessive TRPV3 activity (Asakawa et al., 2006; Xiao et al., 2008). Increased EGFR signaling in keratinocytes also promotes skin cancer. Although increased tumorigenesis has not been described in rodents expressing constitutive active forms of TRPV3, the study by Cheng et al. suggests that this issue deserves additional scrutiny. However, given that Cheng et al. find that loss of *trpv3* does not reduce proliferation, in contrast to the effect of diminished EGFR signaling, it is possible that surplus TRPV3 activity does not lead to skin cancer. Nevertheless, it is possible that TRPV3 has additional roles in the skin, such as in the dermal layer, which underlies the epidermis. Carvacrol, a known TRPV3 agonist, stimulates a Ca^{2+} rise and collagen expression in dermal fibroblasts, and this effect appears to require activation of PLCγ and ERK (Lee et al., 2008). If collagen expression is enhanced by TRPV3, then TRPV3 agonists might represent a new approach for boosting collagen levels in skin.

In addition to TRPV3, other TRP channels, including TRPV1, TRPV4, TRPC6, and TRPA1, are expressed in keratinocytes. These channels may modulate cell proliferation or act in concert with TRPV3 to regulate terminal differentiation in epidermis or hair or to help to maintain the skin barrier. Given the role of TRPV3 in maintenance of the skin barrier, it is possible that activators of TRPV3 and other TRPs would provide a therapy to treat diseases that impair the skin barrier. Consistent with this proposal is the recent finding that agonists for TRPA1, including cinnamaldehyde and cool temperatures, hasten the recovery following damage to the skin barrier (Denda et al., 2010). It would be interesting to test whether TRPV3 activators, such as camphor, have a similar effect, especially in combination with agonists for TRPA1.

ACKNOWLEDGMENTS

C.M. is supported by grants from the NIH. I thank Q. Ren for help in preparing the figure.

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Ubiquitin Gets CARDed

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DOI 10.1016/j.cell.2010.03.047

Ubiquitin-dependent processes are critical for propagating antiviral defense signals during viral infection. In this issue, Zeng et al. (2010) describe how viral RNA and unanchored ubiquitin chains conspire to promote activation of RIG-I and the host cell's antiviral transcriptional program.

Detection of, and defense from, pathogens represents a major challenge for cellular systems, and elaborate mechanisms have evolved to sense common foreign agents and to promote the production of molecules that fight the invader. One such mechanism uses retinoic-acid-inducible gene-I (RIG-I) like receptors (RLRs) to sense viral RNA molecules and initiate a signaling cascade that involves binding of RLRs to the MAVS (mitochondrial antiviral signaling) protein. This leads to MAVS-dependent activation of TANK-binding kinase (TBK1), which phosphorylates interferon regulatory factor 3 (IRF3), promoting its dimerization and translocation to the nucleus. MAVS also promotes activation of Iκ-B kinase (IKK), which promotes the nuclear import of the transcription factor NF-κB. IRF3 and NF-κB then induce transcription of genes involved in antiviral defense (Moore and Ting, 2008). Multiple steps in the pathway, including activation of TBK1 and IKK, as well as nuclear import of NF-κB, are controlled by the ubiquitin system through both regulatory and degradative mechanisms (Bhoj and Chen, 2009). In addition, ubiquitination of RIG-I itself seems to promote the antiviral response (Gack et al., 2007). But how this occurs, how viral RNA serves as a trigger, or how RIG-I signals to MAVS are not clear. Using an elegant in vitro system that recapitulates the major steps in RNA-dependent activation of IRF3 (Zeng et al., 2009), Zeng and colleagues show in this issue of *Cell* that RIG-I activation by RNA promotes recruitment of unanchored lysine 63 (K63)-linked ubiquitin chains to the caspase recruitment domains (CARDs) of RIG-I independent of ubiquitin conjugation to RIG-I (Zeng et al., 2010). This establishes that unanchored ubiquitin chains together with RIG-1 form a potent viral RNA sensor that directly communicates with MAVS to promote IRF3 activation (Zeng et al., 2010), ultimately resulting in an antiviral response.

Polyubiquitin chains linked through specific lysine residues on ubiquitin confer different molecular fates on the conjugated substrate (Ye and Rape, 2009). In response to a viral infection, K63-linked ubiquitin chains serve as scaffolds to activate downstream signaling kinases, including TBK1 and IKK, which activate IRF3 and NF-κB, respectively (Bhoj and Chen, 2009). Upstream of MAVS, the E3 ubiquitin ligase TRIM25 is necessary for interferon-β (IFN-β) production following viral infection, presumably after ubiquitination and activation of RIG-I (Gack et al., 2007). By recreating this signaling cascade in a cell-free system, Zeng et al. (2010) determined the necessary ubiquitin- and viral RNA-dependent triggers required for IRF3 activation. Mixing purified mitochondrial and cytoplasmic fractions from virally infected (but not uninfected) cells resulted in TBK1/IKKε-dependent dimerization of IRF3, a readout for pathway activation (Zeng et al., 2009) (Figure 1). Importantly, this signaling pathway was dependent on RIG-I, MAVS, and TRIM25, as depletion of any of these components blocked or reduced IRF3 activation (Zeng et al., 2010). Transfection of a viral RNA mimic into cells led to robust expression of IFN-β; however, incubation of the same RNA with purified RIG-I failed to activate IRF3 in the in vitro assay. Remarkably, incubation of purified RIG-I with the ubiquitin conjugation machinery and TRIM25, together with viral RNA, restored pathway activation in the absence of viral infection, establishing the critical importance of ubiquitin chain formation for RIG-I activation. Con-