PERSPECTIVE

The incidental pore: Ca_v1.2 and stem cell activation in quiescent hair follicles

Pierre A. Coulombe^{1,2,3,5} and Michael J. Caterina^{2,4,5}

¹Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, USA; ²Department of Biological Chemistry, ³Department of Dermatology, ⁴Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA

The hair follicle undergoes a lifelong developmental cycle that depends on the integration between activating and inhibitory signals acting to regulate and guide the proliferation and differentiation of pluripotent epithelial stem cells. The effectors and mechanisms responsible for re-entry of quiescent telogen hair follicles into the hair-producing anagen stage in mature skin remain incompletely understood. In the June 1, 2013, issue of *Genes & Development*, Yucel and colleagues (pp. 1217–1222) reported the unexpected finding that Ca_v1.2, the pore-forming subunit in a well-characterized voltage-gated, L-type calcium channel, is expressed in hair follicle stem cells and contributes to anagen re-entry but does so in a calcium flux-independent fashion.

L'habit ne fait pas le moine.
[The clothes do not make the man.]
—French proverb

Remarkable progress has been achieved in the last several years in identifying and characterizing progenitor stem cells in organs and tissues of the embryo and adult. Owing to its ready accessibility and lifelong enactment of a developmental cycle that has the dual virtue of recapitulating embryonic events and representing a genuine regeneration paradigm, the adult stage hair follicle (HF) provides a unique opportunity to define and experimentally manipulate stemness, understand its role in human disease (e.g., cancer) and aging, and exploit stem cells for therapeutic purposes. In adult mammalian skin, the HF iterates through a cycle of active growth (anagen), involution (catagen), and quiescence (telogen) (Hardy 1992). At its core, this cycle is made possible by the periodic and limited activation of pluripotent HF (epithelial) stem cells (HFSCs) housed in a specific HF compartment known as the bulge (Cotsarelis et al. 1990). HFSCs feature

many attributes that are currently at the forefront of stem cell research. Examples include a hierarchy of pluripotent stem cell subtypes (e.g., slow cycling vs. rapidly cycling ones), permanent residence within an anatomical niche (the bulge), tight dependency on specialized mesenchymal cells (dermal papillae [DP]) in regulating activity versus quiescence, and several molecular features and strategies that are shared with stem cells in other tissues (Li and Clevers 2010; Fuchs and Chen 2013).

Key to defining whether the adult HF is active versus quiescent is a balance between growth-promoting Wnt signals and quiescence-promoting BMP signals that emanate from the DP (e.g., Plikus et al. 2008; see also Myung et al. 2013). Activation of Wnt signaling is essential for both hair morphogenesis during development and anagen re-entry in the adult HF and is manifested through stabilization of β -catenin in the nucleus of HFSCs (Jamora et al. 2003; Myung et al. 2013). The concomitant attenuation of BMP signaling is essential for the re-entry of quiescent telogen stage HFs into anagen (Fuchs and Chen 2013). This attenuation is brought about in part by the action of DP-derived (and anagen-promoting) TGF- β 2 on HFSCs (Oshimori and Fuchs 2012) and is coupled to activation of Wnt ligands and suppression of Wnt inhibitory signals (e.g., Kandyba et al. 2013). The latter offers insight into how powerful but opposing Wnt and BMP signaling pathways are temporally integrated to generate the crucially important interplay between quiescence and activity in adult HFs. Still, much remains to be deciphered about the effectors and associated mechanisms that orchestrate the shutting down and reactivation of adult HF growth toward the end of anagen and telogen, respectively.

Timothy syndrome (TS; Online Mendelian Inheritance in Man [OMIM] 601005) is a rare, autosomal-dominant condition characterized by severe developmental anomalies (e.g., syndactyly), electrophysiological defects in the heart (e.g., long QT syndrome), and neurological manifestations typical of autism spectrum disorders (Splawski et al. 2004). TS is caused by missense mutations in the Ca_v1.2 (CACNA1C) gene, which encodes the pore-forming α 1C subunit of a voltage-dependent, L-type calcium channel (Splawski et al. 2004). Normally, activation of L-type channels by membrane depolarization results

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⁵Corresponding authors

E-mail coulombe@jhsphe.edu

E-mail caterina@jhmi.edu

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in a sequential and precisely timed transition from a calcium-impermeant closed state to an open state that permits calcium flux into the cell and subsequently to an “inactivated” state, conformationally distinct from the closed state, that nevertheless exhibits little or no calcium influx. The TS-causing mutations impair the voltage-dependent inactivation of $\text{Ca}_v1.2$, resulting in an abnormally sustained inward current response. In the heart, this results in prolongation of action potentials and consequent arrhythmias, which are often fatal (Splawski et al. 2004; Venetucci et al. 2012).

Individuals with TS also feature intriguing defects in ectoderm-derived epithelial appendages, including a marked delay in hair growth during the first 2 yr of life (Splawski et al. 2004). Yucel et al. (2013) explored the link between $\text{Ca}_v1.2$ TS mutations and this hair growth phenotype. They established that a full-length version of the $\text{Ca}_v1.2$ ORF is enriched in the bulge HFSCs of adult mouse HFs. However, when they examined genetically labeled bulge cells in vitro, they were unable to detect depolarization-induced calcium currents or elevations in intracellular calcium.

In an effort to reconcile these findings, Yucel et al. (2013) presented several orthogonal and compelling observations supporting a model in which the $\text{Ca}_v1.2$ protein promotes entry into anagen not by admitting Ca^{2+} ions into bulge cells as expected, but via another path that is unique to the “inactivated” channel state. The investigators showed that activation of $\text{Ca}_v1.2$ in bulge epithelial cells somehow results in the up-regulation of *Fstl1* (follistatin-like1), a soluble antagonist of BMP signaling previously shown to be present in human HSFCs (Ohyama et al. 2006). Impaired inactivation in TS mutant channels prevented up-regulation of *Fstl1*, but this defect could be overcome with dihydropyridine Ca_v regulators that drive channel inactivation, such as verapamil (Yucel et al. 2013). Although the investigators could not exclude a contribution of “gain-of-function” currents below their level of detection, the phenocopying of the TS hair cycle deficits by a $\text{Ca}_v1.2$ mutant that cannot achieve either the open or inactivated state and the inability of verapamil to rescue this phenotype favor an obligate role for the inactivated channel state in the transition to anagen.

Influx-independent conformational changes in $\text{Ca}_v1.2$ have already been proposed to underlie coupling between this channel and ryanodine receptors in cardiomyocytes (McCall et al. 1996). Furthermore, Krey et al. (2013) recently demonstrated that, in neurons differentiated from induced pluripotent stem cells (iPSCs) from TS patients, depolarization drives a $\text{Ca}_v1.2$ -mediated, but calcium influx-independent, dendrite retraction that depends on RhoA. This effect could be antagonized by overexpression of GEM, an RGK family small GTPase that binds to the regulatory β subunit of $\text{Ca}_v1.2$ and can inhibit RhoA signaling. Yucel et al. (2013) suggest that interactions between $\text{Ca}_v1.2$ and GEM or other small GTPases might explain the state dependence of $\text{Ca}_v1.2$'s contribution to HF cycling. They show that the HF bulge expresses GEM and another RGK family GTPase, REM, as well as two other molecules (STIM1 and STIM2)

capable of inhibiting Ca_v ion flux activity. However, loss-of-function experiments in HFSCs will be necessary to address the functional relevance of these potential interactors to the HF phenotype in TS. Nevertheless, if the tantalizing model proposed by Yucel et al. (2013) proves to be correct, an active function of the (apparently misnamed) inactivated $\text{Ca}_v1.2$ state might contribute to certain pathophysiological features of TS across organ systems.

Additional issues of interest are the biochemical events occurring both upstream of and downstream from $\text{Ca}_v1.2$ in HSFCs; namely, the identity and source of the ligand or trigger mechanism responsible for $\text{Ca}_v1.2$ activation, the second messenger pathways engaged by it, and their integration with other signals operating at the telogen-anagen transition. Answering these and related questions will likely provide important and, as is the case for the offering by Yucel et al. (2013), unforeseen insight into HF and stem cell biology.

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