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The Serine Protease Activity of Corin Is Required for Normal Pigment Type Switching

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TO THE EDITOR

The production and deposition of pigment in mouse skin are confined to the hair follicle and hair shaft, respectively. Hair pigmentation in mice involves the deposition of two types of pigment, black (eumelanin) and yellow (pheomelanin), in a very specific pattern (Barsh et al., 2000). Pigment, either black or yellow, is synthesized during the growth phase (anagen) of the hair cycle by melanocytes that reside in the hair bulb surrounding the upper half of the dermal papilla (DP), a specialized mesenchymal compartment that plays important roles in regulating different aspects of hair follicle biology. Keratinocytes in the hair bulb that undergo differentiation to form the building blocks of the hair shaft take up pigment from nearby melanocytes, leading to the formation of pigmented hair.

Activity of Mc1r receptor in melanocytes results in black pigment production (Jackson et al., 2007; Slominski et al., 2005; Smart and Low, 2003). Binding of Agouti to Mc1r reduces Mc1r signaling and switches the production from black pigment to yellow (Ollmann et al., 1998). During early anagen, a sharp peak of *Agouti* expression in DP cells is observed (Millar et al., 1995). This peak generates a short and provisional period in which Mc1r activity is suppressed by Agouti and temporarily switches the melanocytes to produce pheomelanin. This creates a subapical yellow band in an otherwise black hair, resulting in an overall appearance of a mottled brown hair coat.

The interaction between Mc1r and Agouti is modified by Corin (Enshell-Seijffers et al., 2008). *Corin* encodes a type II transmembrane serine protease that is expressed specifically in the DP and adjusts Agouti inhibition by narrowing the window of effective Agouti activity. In the absence of Corin, Agouti activity is prolonged and the yellow band is extended, leading to lighter coat color. Because Corin is a type II transmembrane serine protease and its single-pass transmembrane domain resides in close proximity to the N-terminus, most of Corin is extracellular (Figure 1a). In addition to regulating pigment type switching, Corin plays important role in blood pressure regulation. The protease activity of Corin in the heart cleaves the prohormone Nppa to its active form and thus activates the natriuretic peptide pathway to control blood tension (Chan et al., 2005; Yan et al., 2000). In the uterus, Corin activates Nppa to regulate blood pressure during pregnancy by augmenting trophoblast invasion and remodeling spiral arteries, thus preventing preeclampsia (Cui et al., 2012). In contrast, little is known about the molecular mechanism by which Corin acts to inhibit Agouti activity and regulate pigment type switching.

It was speculated that the protease activity of Corin mediates the Corin role in pigment type switching, but direct evidence for such a mechanism is lacking (Enshell-Seijffers et al., 2008). In contrast to Corin's role in blood pressure regulation, Corin may regulate Agouti activity by a different mode of action that is unrelated to its serine protease activity. In such model, Corin may act as a receptor that transduces signaling into DP cells to modify Agouti activity or as extracellular inhibitor that sequesters the activity of Agouti.

To explore whether the serine protease activity of Corin is required in the regulation of pigment type switching, the serine protease activity of Corin was abolished while preserving its complex structure. Using a gene targeting approach, a missense mutation that substitutes the serine (S) residue of the catalytic triad to alanine (A) was introduced (Figure 1b and c). This alteration has been previously shown in vitro to diminish the catalytic activity of Corin without affecting its surface localization and stability (Yan et al., 2000). Furthermore, this substitution was also designed to introduce the SgrA I restriction site to allow routine genotyping. A targeting construct was generated to include the S/A substitution and transfected into embryonic stem cells. Chimeric mice were derived from correctly targeted embryonic stem cell clones and used to establish a mouse line that harbors the S/A substitution. Wild-type, heterozygous, and homozygous mice for the missense mutation were designated *CorinS/S*, *CorinS/A*, and *CorinA/A*, respectively. Primers that flank the SgrA I site were designed to PCR-amplify a fragment of 390 base pairs (Figure 1b). In the case of the *CorinA* allele, digestion of the PCR product resulted in two fragments of 118 base pairs and 272 base pairs. This way, the *CorinS* and *CorinA* alleles were easily distinguished in agarose gel electrophoresis after digestion with SgrA I (Figure 1d).

Similar to mice with a null allele of *Corin* (Enshell-Seijffers et al., 2008), mice homozygous for the *CorinA* allele were viable, fertile, and recovered at expected Mendelian frequencies. Furthermore, as expected, follicle morphology and the hair cycle appeared normal. Homozygous mutants were also

Abbreviations: A, alanine; DP, dermal papilla; S, serine

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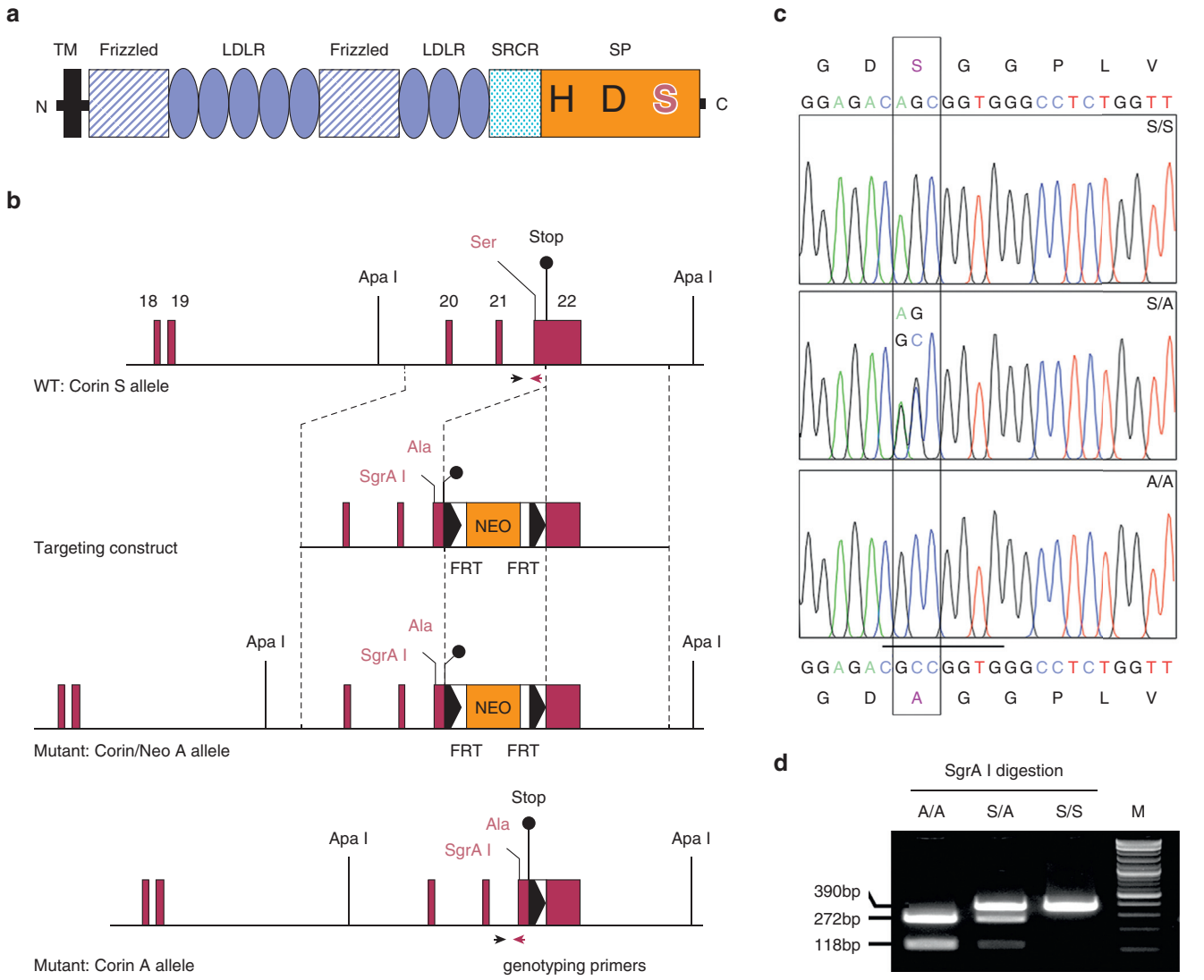

Figure 1. The in vivo generation of a catalytically inactive mutant of Corin. (**a**) A schematic representation of Corin structure is shown. As a type II transmembrane protein, the C-terminus is exposed on the surface of the cell. Note the transmembrane (TM) domain is in close proximity to the N-terminus, and therefore most of the protein is positioned outside the cell. The large extracellular part of Corin includes two cysteine-rich frizzled homology domains, two series of LDLR-like repeats, a scavenger receptor conserved region (SRCR), and a catalytic domain of trypsine-like serine protease (SP) at the C-terminus. The amino acid residues of the catalytic site, histidine (H), aspartate (D) and serine (S) are indicated. (**b**) The gene targeting approach to substitute the S residue to A is shown. The location of the S/A substitution is indicated, and the positions of the genotyping primers are shown as arrows. First, a mouse line with the S/A substitution and a neo cassette was generated. Subsequently, this mouse line was crossed with a mouse line that expresses the FLPe recombinase ubiquitously to delete the neo cassette. (**c, d**) Using the genotyping primers, a PCR fragment was amplified from homozygous wild-type (S/S), heterozygous (S/A), and homozygous mutant (A/A) mice for (**c**) sequencing or (**d**) digestion with SgrA I restriction enzyme. The restriction site for SgrA I is upperlined in the bottom sequence in **c**. A, alanine; bp, base pair; M, marker; WT, wild type.

assessed in vivo for the presence of the Corin mutant in the DP of the hair follicle in skin sections using immunostaining with anti-Corin antibodies (Enshell-Seijffers et al., 2008). Because the missense mutation is not expected to affect Corin levels and structure, these antibodies detected apparently normal levels of Corin in the DP of homozygous *CorinA/A* mutant mice (Figure 2a and b). Phenotypic analysis of coat color on normal *Agouti* background showed that mice homozygous for the *CorinA* allele display a distinctively lighter coat color (Figure 2c–e). Similar to the null phenotype (Enshell-Seijffers et al., 2008), this coat color phenotype is most pronounced in juveniles (Figure 2c). Hair shafts were plucked from the back skin of wild-type and mutant mice at the end of the first hair cycle and analyzed microscopically (Figure 2e). Mice homozygous for the *CorinA* allele exhibit extended subapical yellow band. This clearly illustrates that the serine protease activity of Corin is required for inhibiting Agouti activity during pigment type switching.

A missense mutation in the *Corin* gene of the golden tiger has been recently identified (Xu et al., 2017). This mutation results in the substitution of a histidine residue into tyrosine in the sixth LDLR domain of Corin and therefore does not interfere with its protease activity. However, this genetic alteration results in extremely elongated yellow band and, consequently, a light-colored tiger. Although the ability of Corin to proteolytically cleave Agouti has not been directly tested in that study and therefore precluded the assessment of

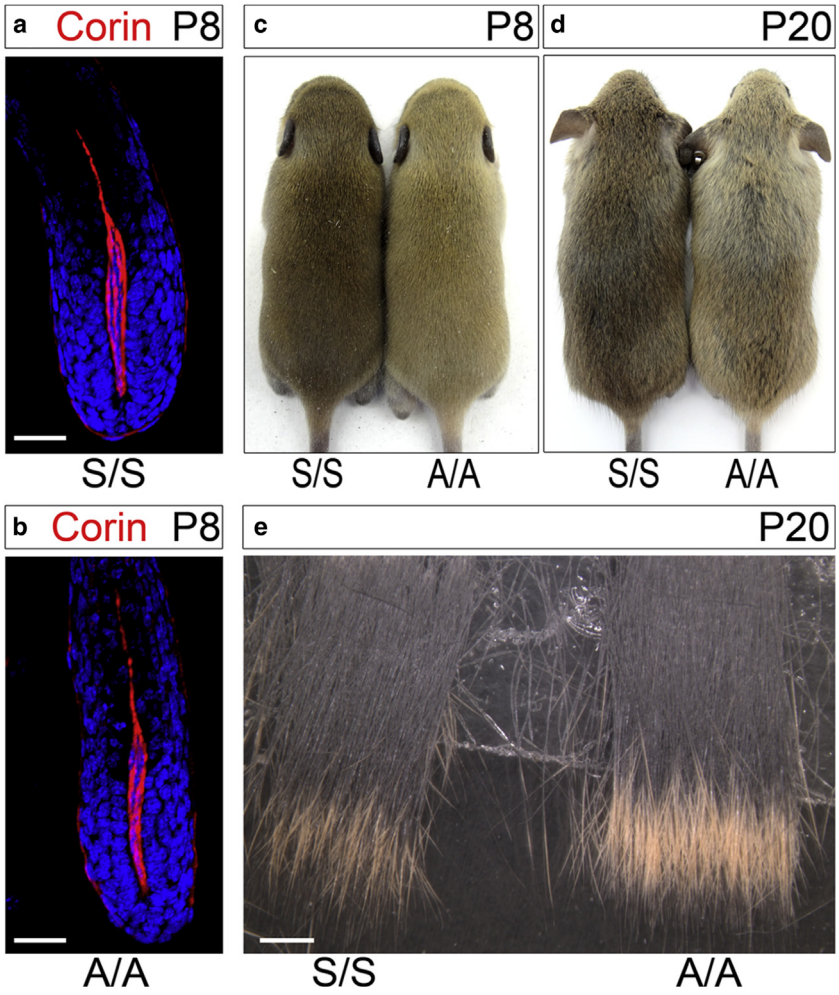

Figure 2. Phenotypic analysis of the catalytically inactive Corin mutant. (**a, b**) Immunostaining of postnatal day 8 (P8) follicles from wild-type (S/S) and mutant (A/A) mice with anti-Corin antibodies shows comparable levels of Corin in the DP. Scale bars = 25 μm. (**c, d**) Mutant mice exhibit lighter coat color than their wild-type littermates. (**c**) An image of wild-type and mutant littermates during the mid-growth phase of the first cycle (P8) is shown. (**d**) An image of wild-type and mutant littermates at the end of the first cycle (P20) is shown. (**e**) Microscopic images of plucked hairs from wild-type and mutant littermates at the end of the first cycle (P20). The subapical yellow band in the mutant is extended. Scale bar = 1 mm. A, alanine; DP, dermal papilla; P, postnatal day; S, serine.

Agouti as a direct substrate of Corin, this analysis may suggest that the underlying mechanism by which Corin interacts with Agouti is similar to the way Corin regulates proANP in the heart (Knappe et al., 2004). Initially, a direct protein-protein interaction between the LDLR domains of Corin and Agouti recruits Agouti to the vicinity of the protease domain, and subsequently, the serine protease activity of Corin inactivates Agouti by proteolytic cleavage. Unfortunately, the low and variable level of Agouti protein in the skin and the limited sensitivity of available antisera prevent the in vivo assessment of direct interaction between Agouti and Corin and the proteolytic consequences of this interaction on Agouti. Only future development of novel tools will allow us to molecularly address the mechanism by which Corin and Agouti interact.

Mice

Mice were housed according to Federation of Laboratory Animal Science Associations guidelines. All mice were bred and maintained in a temperature-controlled room, on a 12-hour light-dark cycle, and with food and water available ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of Bar Ilan University.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.07.024.

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