Development

WNT regulator controls stripe patterning

Colour patterns, such as the stripes on a zebra, reflect the precise spatiotemporal control of gene regulation and thus are informative for understanding the mechanisms underlying animal development. In a recent *Nature Ecology & Evolution* paper, Johnson et al. investigate the alternating light and dark dorsal stripes of the African striped mouse *Rhabdomys pumilio*. Using a combination of functional genomics, gene editing and mathematical modelling, the authors demonstrate how a WNT regulator contributes to the alternating light and dark dorsal stripes and overall pigmentation of these rodents.

Johnson et al. first observed that the hairs from the darker stripes were shorter than the hairs from the lighter stripes, which suggested that the developmental timing of hair follicle placodes (that is, embryonic structures) might differ between stripe regions. In support of their hypothesis, in situ hybridization of early-placode markers showed that hair follicle placodes from eventual dark hair stripe regions indeed form later than those from light hair stripe regions.

To identify factors regulating the timing of placode formation, the authors performed bulk RNA sequencing (RNA-seq) on dissected tissue and compared gene expression levels between the light and dark stripes. The most consistently differentially expressed gene between stripes was *Sfrp2*, a modulator of the WNT signalling pathway, which is a primary determinant of hair follicle formation. To understand how *Sfrp2* regulates WNT signalling in these stripes, the team combined single-cell RNA-seq with multiple RNA visualization approaches and concluded that *Sfrp2* likely negatively regulates WNT signalling expression in striped mice dermal fibroblasts. Furthermore, mathematical modelling supported a mechanism that reconciled *Sfrp2* negatively regulating WNT signalling with the observed expression pattern of *Sfrp2* across the stripes.

With the descriptive data implicating *Sfrp2* in colour stripe patterning, Johnson et al. next performed transgenic and gene-editing experiments to functionally validate this mechanism. First, they expressed a *Sfrp2* transgene in the skin of laboratory mice and showed that elevated levels of *Sfrp2* caused a decrease in hair follicle placodes. Second, they used CRISPR–Cas9 to abolish *Sfrp2* function in African striped mice, demonstrating successful in vivo gene editing in a wild-derived mammalian species. Characterization of *Sfrp2*-deficient striped mice found that stripe width depended on *Sfrp2* function. Moreover, these mice also had overall lighter pigmentation, suggesting that *Sfrp2* regulates WNT signalling at multiple points in development.

Having detailed the role of *Sfrp2* in stripe formation, the authors next sought insight into the evolution of this developmental mechanism. They first compared the orthologous coding sequences of *Sfrp2* between many mouse species; on the basis of substitution rate and tests of positive selection, they found no evidence implicating *Sfrp2* coding changes in the evolution of striped patterns. This finding was consistent with their data on the *Sfrp2* expression patterns in African striped mice that mirrored the dorsal stripes, suggesting that genetic changes contributing to this mechanism more likely occurred in *cis*-regulatory elements (CREs) than in coding regions. To identify potential causative CRE changes, the authors performed ATAC-sequencing on embryonic dorsal tissue from laboratory mice and used open chromatin regions near *Sfrp2* as a proxy for putative CREs controlling *Sfrp2* expression. After aligning putative CREs from multiple mouse species, they found multiple genetic changes unique to African striped mice that are predicted to alter transcription factor binding and thus, potentially, leading to spatiotemporal differences in *Sfrp2* expression.

> **"...*Sfrp2* likely negatively regulates WNT signalling expression in striped mice dermal fibroblasts"**

Through an integrative approach involving varied data types, Johnson et al. have identified a developmental mechanism underlying the distinct colour patterning of African striped mice. In doing so, they have illustrated the interesting biology yet to be explored in non-model organisms.

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