DEVELOPMENT

Staying on course with PIAS3

In the course of differentiation, retinal rod photoreceptors must both repress the expression of cone-specific genes and activate the expression of rod-specific genes. These processes are regulated by rod-specific transcription factors, including NR2E3, but how these transcription factors can both activate and repress gene transcription has remained unclear. Now, Onishi *et al.* show that in differentiating rods the transcriptional co-regulator PIAS3 suppresses cone-specific gene expression by SUMOylating NR2E3.

> “...SUMOylation of NR2E3 is crucial for repressing cone-specific genes...”

PIAS3 expression levels in the developing mouse retina have been shown to be highest during the period of rod specification, indicating that PIAS3 might regulate this process. Indeed, overexpression of PIAS3 in developing retinas through *in vivo* electroporation increased the number of retinal cells that had a rod-like morphology and that expressed the rod-specific protein rhodopsin. Conversely, reducing PIAS3 expression using short hairpin RNA increased the number of cells that had a cone-like morphology and that co-expressed both rod- and cone-specific markers.

How does PIAS3 influence the expression of photoreceptor-specific genes? Immunoprecipitation experiments demonstrated that it interacts directly with the photoreceptor-specific transcription factor NR2E3. The importance of this interaction for rod development was demonstrated in retinas from animals lacking NR2E3: here, PIAS3 overexpression no longer increased the number of rod cells, and *Pias3* short hairpin RNA was much less potent at inducing cone-like cells than in wild-type retinas.

These findings indicated that PIAS3, through its interaction with NR2E3, regulates the expression of rod- and cone-specific genes. Indeed, chromatin-immunoprecipitation experiments using antibodies against PIAS3 and NR2E3 showed that both factors bind to the promoter regions of rod- and cone-specific genes.

PIAS3 can act as an E3 SUMO ligase, and to ascertain whether it SUMOylates NR2E3 the authors transfected HEK293T cells with NR2E3, Flag-tagged SUMO1 and either wild-type PIAS3 or a mutant form of the protein that lacks the E3 SUMO ligase activity. Wild-type PIAS3 indeed SUMOylated NR2E3. *In vivo*, overexpression of the mutant PIAS3 increased the number of cells expressing cone-specific markers but had no effect on the number of rhodopsin-expressing cells, suggesting that the SUMOylation function of PIAS3 is important for preventing rod precursors from developing into cones. Specifically, the SUMOylation of NR2E3 is crucial for repressing cone-specific genes: mice lacking NR2E3 had an abnormal retinal phenotype (with more cone-like cells) that could be rescued by electroporation of wild-type or SUMO1-fused NR2E3 but not SUMOylation-deficient forms of NR2E3. However, rhodopsin expression was unaffected by SUMOylation-deficient NR2E3, suggesting that NR2E3 SUMOylation is not required for inducing rod-specific gene expression.

Nevertheless, chromatin-immunoprecipitation experiments showed that both rod- and cone-specific gene promoters in rods are bound by SUMOylated proteins to a greater extent than non-photoreceptor-specific promoters. Moreover, a general inhibition of SUMOylation in developing retinas caused photoreceptors to adopt a cone-like phenotype and to not express rhodopsin. This indicates that SUMOylation does have a role in promoting rod-specific gene expression but that this probably involves PIAS3-mediated SUMOylation of a protein other than NR2E3.

Thus, in rod photoreceptor precursor cells, PIAS3 selectively SUMOylates NR2E3 bound to the promoters of cone-specific genes, resulting in the repression of these genes and allowing differentiation into rods. These findings imply that, in addition to transcription factors, transcriptional co-regulators can be key determinants of neuronal cell-fate specification and that SUMOylation can coordinate both positive and negative regulatory events in the differentiation of a single neuronal subtype.

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ORIGINAL RESEARCH PAPER Onishi, A. *et al.* Pias3-dependent SUMOylation directs rod photoreceptor development. *Neuron* **61**, 234–246 (2009)