

# When Pigment Cells Turn Into Neurons

Kapil Bharti and Heinz Arnheiter

Mammalian Development Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

The phenomenon that one cell type can be converted into another has recently generated much excitement because it promises to make cell-based therapies feasible. If we manage to take skin cells, for instance, and turn them into insulin-producing  $\beta$  cells, dopaminergic neurons, or glia, we would have a cure for many of the ailments that can render our lives miserable. But what are the mechanisms that guide such cellular interconversions?

A basic framework to understand how cells change their phenotypes is provided by the mechanisms operating during development. The development of a cortical neuron, for instance, occurs by a complex multistep process involving extracellular signals and the consecutive action of partly overlapping sets of transcription factors. Among these, one set keeps neural precursors (cells capable of generating neurons and glia) in a proliferating stage, another one commits them to the neuronal fate, and a third imparts neuronal subtype-specific characteristics (Ross *et al*, 2003). It would seem, therefore, that the best way to obtain a particular functional cell type is to start out with the least differentiated cell and recapitulate normal development. But, given that some transcription factors such as MYOD or PAX6 are capable of changing single handedly the phenotypes of many different cell types (Boukamp, 1995; Halder *et al*, 1995), it is tempting to shortcut the process of cellular interconversions by simply force-expressing such regulators in undifferentiated or even fully or partially differentiated cells. The latter is what Lanning *et al* (2005) did when they used a transgenic mouse approach to express a neurogenic transcription factor, mammalian achaete-scute homolog-1 (MASH1), under the control of a tissue-restricted regulatory element in cells that normally turn into pigment cells. The authors find that in response to MASH1, one type of pigment cells, retinal pigment epithelium (RPE) cells, initiates neurogenesis whereas another, melanocytes, does not demonstrably change cell fate and shows differences only in coloration. These findings raise interesting conceptual and technical questions.

The combination of MASH1 and pigment cells to test cell type conversions is attractive for several reasons. MASH1 normally serves to promote neuronal differentiation both in the central and peripheral nervous system (Ross *et al*, 2003; Howard, 2005). It belongs to the basic-helix-loop-helix

class of transcription factors that form homo- and heterodimers and specifically bind E box motifs in target promoters. Interestingly, pigment cells likewise depend on a dimerizing E box-binding transcription factor, microphthalmia-associated transcription factor (MITF), a member of the basic-helix-loop-helix-leucine zipper class of proteins (Hodgkinson *et al*, 1993). Importantly, however, the two proteins do not form DNA-binding heterodimers with each other and share little interest in each other's favorite E boxes, and so it is unlikely that MASH1 would compete directly with MITF for access to its target genes.

RPE cells are derived from the optic neuroepithelium—which is part of the central nervous system—and they form a layer at the back of the eye where they absorb light. Melanocytes are derived from the neural crest—which in addition gives rise to the peripheral nervous system—and they populate various sites including iris, choroid, inner ear, and skin. Thus, both types of pigment cells have their origin in neurogenic tissue but neither is normally neurogenic itself. Moreover, RPE cells form a contiguous monolayer as does the early neuroectoderm, and so the mechanisms of fate determination in the RPE may resemble those leading to the regionalization of the neuroectoderm. In contrast, melanoblasts, the precursors to melanocytes, are well separated, individual cells, and so their mechanisms of differentiation may resemble more closely those specifying individual neurons.

The fundamental differences between the two types of pigment cells suggest a possible explanation for why MASH1 is able to convert only one of them into neuronal tissue. Melanoblasts, in order to reach their final destination, have to migrate through many different cellular environments that may induce fluctuations in gene expression, including the expression of neurogenic factors. Hence, they may have been selected evolutionarily not to succumb to such fluctuations and change their fate too willingly. By comparison, RPE cells have a stable relationship with their neighbors, face a constant environment, and may not have evolved a similar resistance to fate-changing insults that they may not normally encounter.

Although the above considerations focus on cellular and molecular interpretations, the results can also be explained on more technical grounds. Transgenic studies always present us with the challenge of how best to control transgene expression spatially and temporally. To ensure that MASH1 is specifically made in cells that have already initiated differentiation towards the pigment lineage, Lanning *et al* (2005) link a MASH1 cDNA to the regulatory region of

---

Abbreviations: Dct, dopachrome tautomerase; MASH1, mammalian achaete-scute homolog-1; MITF, microphthalmia-associated transcription factor; RPE, retinal pigment epithelium

the *dopachrome tautomerase (Dct)* gene which encodes an enzyme involved in pigment biosynthesis. Evidently, when the cells switch their phenotype, they lose the expression of pigment genes. Indeed, transgenic MASH1 suppresses several pigment gene promoters including the *Dct* promoter, and hence it suppresses its own *Dct*-driven expression, leading to unpredictable and variable levels of MASH1. Therefore, potential quantitative differences between RPE cells and melanocytes in this negative feedback loop could explain why the two cell types respond differently to MASH1. To overcome this problem, one may have to employ binary transgenic systems such as those based on the Cre/loxP system, which allows one to first activate gene expression cell specifically but in a way that it then becomes cell type independent and permanent. It is also important to consider how to achieve accurate dynamic control of expression. MASH1 is found at low levels in neural progenitors, at higher levels when they start to differentiate into neurons, and at lower levels again when they become terminally differentiated. To mimic experimentally such precise temporal control of expression may require combinations with drug-mediated regulation. Lastly, regardless of whether there are inherent feedback loops, plasmid-based transgenes are notorious for their cell-to-cell variation in expression. To overcome this problem, engineered bacterial artificial chromosomes or gene targeting (knock-ins) may have to be used, as they allow better insulation against such variabilities.

The report by Lanning *et al* (2005) provides a glimpse of the mechanism by which transgenic MASH1 leads to the conversion of RPE into neural tissue. The authors show that MASH1 downregulates MITF, and it has been previously demonstrated that the downregulation of MITF activity alone promotes an RPE-to-retina transition (Galy *et al*, 2002; Rowan *et al*, 2004; Horsford *et al*, 2005). In fact, eyes of *Mitf* mutants, including null mutants, sport a fully laminated second retina adjacent to the normal one (Nguyen and Arnheiter, 2000). Although transgenic MASH1 is not able to change RPE all the way to a retina, one might argue that by whatever mechanism MITF is downregulated, the RPE will always at least initiate the conversion to neuronal tissue. It is important to show, therefore, that downregulation of MITF by MASH1 is indeed because MASH1 is neurogenic, as one could imagine that the unphysiological expression of many proteins, neurogenic or not, might have a similar effect on MITF.

The careful histological analysis of the conversion of RPE into neural tissue reveals an additional subtlety worth mentioning. Both *Mitf* mutations (Nguyen and Arnheiter, 2000) and MASH1 expression lead to neural formation only in a limited section of the RPE. In *Mitf* mutants, conversion usually involves the dorsal part of the RPE (Nguyen and Arnheiter, 2000). In MASH1 transgenics, it involves the anterotemporal region. In the transgenics, one might explain

this by the peculiarities of mosaic transgene expression, but in *Mitf* null mutants, all cells are equally mutant, and so mosaicism cannot be the reason. Rather, we would argue that both RPE conversion, and the resistance to it, requires the cooperation of multiple extrinsic and intrinsic factors. For instance, conversion could be helped dorsally by dorsal signals and transcription factors such as Tbx5 (Koshiba-Takeuchi *et al*, 2000); and it could be inhibited ventrally by ventral signals and transcription factors such as VAX1 and VAX2 (Mui *et al*, 2005). This reasoning prompts one to set up specific matings between MASH1 transgenics and mice carrying different transgenes or mutations in order to delineate the partners with which MASH1 has to collaborate for more efficient cellular conversions.

In conclusion, the report by Lanning *et al* (2005) shows that the neurogenic MASH1 protein can change the fate of one cell type, RPE, but not that of another, melanocytes. From a different angle than previous studies, the paper illustrates how dissimilar these two cell lineages are. It is through such studies that we ultimately gather the knowledge that may lead to a readily harvestable, universal donor cell type that for cell-based therapies could be tailored to the needs of individual patients.

---

DOI: 10.1111/j.0022-202X.2005.23876.x

## References

- Boukamp P: Transdifferentiation induced by gene transfer. *Semin Cell Biol* 6: 157–163, 1995
- Galy A, Neron B, Planque N, *et al*: Activated MAPK/ERK kinase (MEK-1) induces transdifferentiation of pigmented epithelium into neural retina. *Dev Biol* 248:251–264, 2002
- Halder G, Callaerts P, Gehring WJ: Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* 267:1788–1792, 1995
- Hodgkinson CA, Moore KJ, Nakayama A, *et al*: Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* 74:395–404, 1993
- Horsford DJ, Nguyen MT, Sellar GC, *et al*: Chx10 repression of *Mitf* is required for the maintenance of mammalian neuroretinal identity. *Development* 132:177–187, 2005
- Howard MJ: Mechanisms and perspectives on differentiation of autonomic neurons. *Dev Biol* 277:271–286, 2005
- Koshiba-Takeuchi K, Takeuchi JK, Matsumoto K, *et al*: Tbx5 and the retinotectum projection. *Science* 287:134–137, 2000
- Lanning JL, Wallace JS, Zhang D, *et al*: Altered melanocyte differentiation and RPE transdifferentiation induced by *Mash1* expression in pigment cell precursors. *J Invest Dermatol* 125:805–817, 2005
- Mui SH, Kim JW, Lemke G, *et al*: Vax genes ventralize the embryonic eye. *Genes Dev* 19:1249–1259, 2005
- Nguyen M, Arnheiter H: Signaling and transcriptional regulation in early mammalian eye development: A link between FGF and MITF. *Development* 127:3581–3591, 2000
- Ross SE, Greenberg ME, Stiles CD: Basic helix-loop-helix factors in cortical development. *Neuron* 39:13–25, 2003
- Rowan S, Chen CM, Young TL, *et al*: Transdifferentiation of the retina into pigmented cells in ocular retardation mice defines a new function of the homeodomain gene Chx10. *Development* 131:5139–5152, 2004