though these neuronal precursors were rostral to the region of *Fgf8* expression. This resulted in a striking reduction in numbers of mesDA neurons and generation of an equivalent number of 5-HT-containing neurons [25] (Figure 2). These data indicate that Otx2 is required to provide midbrain neuronal precursors with a specific differentiation code, suppressing that of the rostral hindbrain by conferring competence for interpreting Shh and FGF8 signaling activities.

Conclusions and perspectives

Mouse models here discussed are converging to elucidate genetic control of differentiation of the mesDA system. In this context a single gene, *Pitx3*, is required for the terminal differentiation of the SN subpopulation of mesDA neurons. Importantly, the *ak* mutant model exhibits phenotypic features in common with human Parkinson's disease, and it could be a powerful tool for studying molecular and behavioural aspects of the mesDA system, as well as for generating novel pharmacological approaches. Such knowledge might also contribute to the generation of genetically modified stem cells capable of directing generation of dopaminergic neurons equivalent to those of the SN.

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Research Focus Response

Response to Simeone: Coexpression of Pitx3 with tyrosine hydroxylase in midbrain dopaminergic neurons

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In his Research Focus article [1], Antonio Simeone highlights the distinct role of the homeobox transcription factor Pitx3 in terminal specification of midbrain dopaminergic neurons, as revealed in the loss of dopaminergic

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neurons in the substantia nigra (SN) of the natural mouse mutant aphakia (ak). It is pointed out that 'This analysis [of Pitx3 expression in tyrosine hydroxylase (TH)-containing neurons] has provided contrasting results…This apparent difference has yet to be resolved.'

This point is indeed crucial because the Pitx3-expressing neurons are affected when Pitx3 is absent, either by loss at terminal differentiation in the SN or by morphological impairment in the ventral tegmental area (VTA). Partial expression of Pitx3 in the population of midbrain dopaminergic neurons, as reported by Van den Munckhof *et al.* [2], could differentially equip midbrain dopaminergic neurons with susceptibility to downstream effects of Pitx3. These authors exploited this aspect to propose a relationship with Parkinson's disease; they addressed the coexpression of Pitx3 and TH by double immunocytochemistry and reported that Pitx3 is differentially localized in the ventral SN and in ~50% of the VTA TH-positive neurons.

In our study [3], we observed no TH-containing neurons that lacked expression of Pitx3 when we examined multiple subregions of the adult SN and VTA using double *in situ* hybridization and immunocytochemistry, in both combinations (TH antiserum with Pitx3 *in situ* probe, and TH *in situ* probe with Pitx3 antiserum). We observed TH expression without Pitx3 expression only in other, unrelated dopaminergic neurons (those of the hypothalamus and olfactory bulb) and in noradrenergic neurons [4,5].

Here, one could discuss technicalities but, interestingly, a genetic experiment has recently solved this discrepancy. The group of Meng Li generated a mouse line with green-fluorescent protein (GFP) inserted into the Pitx3 locus [6] and visualized Pitx3-directed GFP expression in dopaminergic neurons. In adult heterozygous Pitx3-GFP mice, Pitx3-directed expression of GFP recapitulated Pitx3 expression. Notably, the vast majority of Pitx3-GFP-expressing neurons were TH-positive, and vice versa. Cell counts showed that 98% and 95% of TH-positive cells were Pitx3-GFP-positive in SN and VTA, respectively. Conversely, 99% and 95% Pitx3-GFP-positive cells were TH-positive. In fetal mice at embryonic day (E)12, Pitx3-GFP-expressing and TH-expressing domains partially overlap, with only 56% of Pitx3-GFP-positive cells coexpressing TH. This was not the case at E14. Li and colleagues suggest that the developmental induction of Pitx3-GFP expression precedes that of TH in some midbrain dopaminergic progenitors [7] (M. Li, personal communication).

Taken together, it should be concluded that at least 95% of dopaminergic neurons in the SN and VTA coexpress Pitx3 and TH. Thus, both proteins are part of the total mesencephalic dopaminergic lineage. However, other genes and mechanisms must exist that differentiate between the dopaminergic neurons of the SN and those of the VTA [7]. The absence of dopaminergic neurons in the SN and the selective degeneration of dopaminergic neurons in the SN in Parkinson's disease reveal these differences in a dramatic way. The discriminating factor is not Pitx3 itself, but one or more of its downstream targets. Pitx3 could thus serve as a key to open the door to the differential molecular mechanisms of differentiation, survival and maintenance of SN and VTA dopaminergic neurons.

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