Development of the dopaminergic neurons in the rodent brainstem

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Abstract

The loss of dopaminergic (DA) neurons in the ventral midbrain is the principal cause of Parkinson's disease. The search for candidate molecules that promote the genesis and survival capacities of DA neurons is a major area of investigation and hope. A better characterization of the developmental pathways that govern the specification, differentiation, and survival of these neurons will be essential in devising therapies aimed to rescue or replace midbrain DA neurons in Parkinson's patients. In this brief review, we will discuss the major steps in the normal development of midbrain DA neurons.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder caused by the progressive loss of midbrain dopaminergic (DA) neurons that innervate the striatum (Lang and Lozano, 1998a,b). It is characterized clinically by tremor, rigidity, bradykinesia and postural instability. The cause of the selective loss of DA neurons is still unknown, although several mechanisms have been proposed such as increased oxidative stress, mitochondrial dysfunction or excitotoxic damage, among others (Lang and Lozano, 1998a,b; Olanow and Tatton, 1999). Current approaches used in the treatment of PD include symptomatic treatment with combined L-DOPA and carbidopa, which increase the synthesis and release of dopamine and are particularly effective at alleviating the akinesia and the rigidity during the early stages of the disease. However, as the disease progresses, fewer DA neurons are available to synthesize dopamine. A therapeutic approach to PD assayed for the last decades has been the implantation of dopamine-producing cells in the striatum (Yurek and Sladek, 1990). Among the various classes of DA cells used, the best results, both in animal models of PD and in patients, have been obtained with fetal midbrain neurons (Olanow et al., 1996; Lindvall and Hagell, 2000; Dunnett et al., 2001; Redmond et al., 2001). However, the success of this approach has been limited by practical and ethical issues associated with the need for six or seven human fetuses to provide sufficient numbers of DA neurons for one PD patient. Because a high number of DA cells are required to achieve therapeutic effects, the approach of engineering a complete midbrain DA phenotype in multipotent stem/neural progenitor cells has received considerable attention. To achieve this goal, it is necessary to understand the cell and molecular events that are normally involved in the specification, differentiation and the survival

of midbrain DA neurons. We will review recent progress in efforts to pinpoint key events and factors necessary for the normal development of midbrain DA neurons.

Anatomical organization of midbrain DA neurons

In the adult CNS nearly 75% of all DA neurons reside in the ventral part of the midbrain and are concentrated in three distinct areas: the retrorubral field (A8), the substantia nigra pars compacta (A9, SNc) and the ventral tegmental area (A10, VTA) (Dahlstrom and Fuxe, 1964; Foster, 1994; Kitahama et al., 1994; Reiner, 1994). These three groups assume distinct functions and project to specific brain regions. The A9 neurons project to the dorso-lateral striatum through the nigrostriatal pathway and to the cerebral cortex through the mesolimbicortical system. The projections of the A10 neurons follow the medial forebrain bundle to the ventro-medial striatum and the cerebral cortex as part of the mesolimbicortical system that is involved in emotional behavior and mechanisms of reward. As for the axons of the A8 neurons, some are mixed with the nigrostriatal projections, while others send projections towards the SNc and VTA and appear to be involved in the interconnection of the SN and VTA.

Birth date of midbrain DA neurons

Dopaminergic neurons in A9 are generated during a period extending from E12 to E16 in rats (E10-E14 in mouse) with a peak of genesis at E13 (Lauder and Bloom, 1974). A gradient in the genesis of these neurons has been reported, such that the more anterior and dorso-lateral neurons are generated earlier than the posterior and ventro-medial cells (Bayer et al., 1995). Dopaminergic neurons populating the retrorubral field

and the VTA are generated at roughly the same time, with substantial proportions (49% and 37% respectively) born before E11 in the mouse (Bayer et al., 1995). In all vertebrate species studied, DA neurons begin to express the dopamine rate-limiting synthesis enzyme, tyrosine hydroxylase (TH), soon after the end of their last mitosis, and while they are actively migrating to their final positions (Puelles and Medina, 1994, in chicks; Specht et al., 1977a,b in rats; Puelles and Verney, 1998 in humans).

Early specification of DA progenitors

The early development of DA neurons in the ventral midbrain relies on the establishment of appropriate dorso-ventral and antero-posterior patterns of gene expression. These cells are generated in the immediate vicinity of two organizing centers, the floor plate in the ventral midline and the isthmus at the midbrain/hindbrain boundary. The main dorso-ventral morphogenetic molecule is sonic hedgehog (Shh), a diffusible factor produced in the floor plate. The antero-posterior pattern is established by a more complex interplay of genes that forms the isthmic organizer or the midbrain-hindbrain organizer (MHO), a structure necessary and sufficient for the development of the midbrain and hindbrain. The MHO is induced and controlled by the expression of two homeodomain transcription factors: Otx2 and Gbx2. The importance of the positioning of the MHO has been demonstrated by the production of several transgenic mouse lines in which the shifting of MHO either caudally (Otx2 ectopic expression in the hindbrain) or rostrally (Otx1 and Otx2 drastic depletion in the midbrain) leads to an increase or a decrease in the DA neuronal population, respectively (Brodski et al., 2003). Two secreted factors, Wnt1 and FGF8, are produced in the developing MHO, and several studies have demonstrated that DA progenitors are specified by the

combined action of Shh and FGF8 (Hynes et al., 1995a,b; Ye et al., 1998; Hynes and Rosenthal, 1999). The paired-domain protein Pax2 is necessary and sufficient for the induction of FGF8. A network of transcription and secreted factors, including En-1, Otx2, Gbx2 and Wnt-1, established independently of Pax2, further refine the expression domain and level of FGF8 at the MHO through opposing effects on Pax2 activity (Ye et al., 2001). The inductive signal of Shh is dominant in this event, since the overexpression of Gli1, a downstream effector of Shh, can ectopically induce the expression of DA cell-markers (Hynes et al., 1997). Ectopic expression of Shh or Gli1 in dorsal midbrain areas expressing Fgf8 will also result in ectopic DA neurons. Olfactory and diencephalic DA neurons also originate in overlapping expression patterns of Shh and FGF8 (Ye et al., 1998).

Members of the Wnt family of secreted glycoproteins are also expressed in the midbrain (Parr et al., 1993) and are known to regulate precursor proliferation, fate decisions, and neuronal differentiation. Interestingly, genetic invalidation of Wnt-1 or LRP6 (low-density lipoprotein receptor-related protein) a receptor necessary for Wnts signalling results in the loss of midbrain DA neurons (Danielan and McMahon, 1996; Pinson et al., 2000). Recently, the contribution of Wnts has been elucidated *in vitro*. Wnt-1 controls the proliferation of Nurr1 precursors and increases the number of DA neurons (Castelo-Blanco et al., 2003). Wnt-3a enhances the proliferation of Nurr1 precursors, but prevents the differenciation of DA neurons. By contrast, Wnt-5a increases the number of DA neurons by regulating the acquisition of DA phenotype (Castelo-Blanco et al., 2003). Combined, these findings suggest an important role for Wnts in regulating midbrain DA development. The transforming growth factor (TGF)-β that is produced by the notochord and floor plate has recently been shown to be required for the induction of midbrain DA neurons (Farkas et al., 2003). Thus, i*n vitro*

application of TGF-β has been found to increase the number of TH-positive neurons arising in floor plate explants and, conversely, neutralization of TGF-β prevents the emergence of TH immunoreactivity, suggesting that TGF-β is necessary for Shh to induce TH-positive neurons. Interestingly, blockade of TGF-β during the late phase of midbrain DA development leads to a significant loss of dopamine-containing neurons, which suggests that this growth factor plays a role in the maintenance and survival of these neurons.

Induction and maintenance of DA neurotransmitter phenotype and survival of DA neurons

Unlike the genes mentioned above which are involved in the specification of DA progenitors, recent studies have identified several genes that are required for the development and the maintenance of the DA neurotransmitter phenotype and the survival of DA neurons: the nuclear receptor Nurr1, and the homeodomain transcription factors Ptx3, Lmx1b, En-1 and En-2.

Nurr1 and the neurotransmitter phenotype

Nurr1 is a member of the nuclear receptor superfamily of ligand-activated transcription factors (Law et al., 1992). In mice, Nurr1 is expressed in the midbrain from E10.5, just prior to the appearance of TH (Zetterstrom et al., 1996). Nurr1 is not restricted to midbrain DA neurons as it is also expressed in A11 and A2 catecholaminergic cells (Baffi et al., 1999). Deletion of Nurr1 in mice results in the loss

of expression of TH and c-ret, whereas A11 and A2 catecholaminergic neurons are not affected (Zetterstrom et al., 1997; Castillo et al., 1998). Experiments using E12.5 Nurr1 knockout mice show that in the absence of Nurr1, DA precursors adopt a normal ventral localization and neuronal phenotype characterized by the expression of Pitx3 (Saucedo-Cardenas et al, 1998). In gain-of-function experiments, it was shown that Nurr1 is able to activate the transcription of the TH gene by binding a responsive element within a region of the TH promoter necessary for midbrain specific expression. Interestingly, the action of Nurr1 upon DA neurons has been shown to be independent of FGF8 and Shh signalling (Sakurada et al., 1999). Recently, it has been demonstrated that Nurr1 induces the expression of specific DA markers, the vesicular monoamine transporter and the plasma membrane dopamine transporter (Smits et al., 2003).

Maintenance and survival of DA neurons

The bicoid-related homeodomain transcription factor Ptx3/Pitx3 is expressed in the mouse ventral midbrain by E11.5, just after Nurr1 expression (Semina et al., 1998; Smidt et al., 1997). Pitx3 is exclusively and protractedly expressed in midbrain DA neurons in rodents and humans (Smidt et al., 1997). Recently, the analysis of mice lacking Pitx3 has shown the specific requirement of this gene for the development of the DA neurons of the SNc (Nunes et al., 2003). Surprisingly, Pitx3 is not essential for the induction of the DA neurotransmitter phenotype and the expression of genes involved in midbrain DA neurons like Nurr1, Lmx1b, En1/2, and cRET. Targets of Pitx3 have not yet been described.

Lmx1b is a member of the LIM-homeodomain family of transcription factors. It is expressed as early as E7.5 in the mouse ventral neural tube including the

presumptive region of the midbrain. Mice lacking Lmx1b (Chen et al., 1998) show a loss of midbrain DA neurons, although early expression of Nurr1 and TH is observed. Interestingly, Pitx3 is not induced in these DA neurons. This shows that Lmx1b is necessary for the survival of midbrain DA neurons (Smidt et al., 2000).

Engrailed, a homeodomain transcription factor, has well defined roles in insect development, establishing the body plan. Vertebrates have two engrailed homologs, En-1 and En-2, which are expressed during development in a domain encompassing the posterior midbrain and anterior hindbrain (Gardner et al., 1988). En-1 is highly expressed by essentially all midbrain DA neurons, whereas En-2 is expressed by a subset of them (Simon et al., 2001). In En-1/En-2 double null mutants DA neurons are generated and differentiate their DA phenotype, but disappear soon thereafter (Simon et al., 2001) indicating that engrailed genes control the survival of midbrain DA neurons. Interestingly, additional findings indicate that En-1 and En-2 regulate expression of α-synuclein, a gene that is linked to PD (Simon et al., 2001).

Together, these data suggest that at least three molecular cascades operate during the early differentiation of midbrain DA neurons: one necessary for the induction of the neurotransmitter phenotype and the others for their maintenance and survival (Fig. 1; Table 1). Interestingly, these transcription factors are expressed protractedly in midbrain DA neurons. This has prompted the suggestion that they may not be solely involved in the differentiation of these neurons, but they could participate in the maintenance of the DA phenotype and/or survival of discrete midbrain DA neuronal populations.

Migration of embryonic midbrain DA neurons

Midbrain DA neurons follow a bi-phasic mode of migration (Fig. 2, Table 1). Using TH labeling together with BrdU immunoreactivity, Kawano et al. (1995) have shown that DA cells, generated before E11, first migrate ventrally along radial glial processes expressing tenascin. Then, they migrate laterally in close apposition with tangentially oriented fibers to form the VTA and the winged shaped SNc (Shults et al., 1990; Kawano et al., 1995). The neural cell adhesion molecule L1 is expressed on tangentially oriented fibers, whereas midbrain DA neurons express a ligand of L1/NgCAM, the chondroitin sulfate proteoglycan 6B4. It has been proposed that the lateral migration of DA neurons may be controlled by heterophilic interactions between L1 and 6B4 (Ohyama et al., 1998). As a consequence, mice lacking L1 display positional alterations of midbrain DA neurons (Demyanenko et al., 2001). Furthermore, abnormal routing of tangentially oriented L1 fibers, such as those observed in $Pax6^{-/-}$, induces alterations in the position of DA neurons (Vitalis et al., 2000), further demonstrating the role of L1 expressing fibers in the late guidance of migrating DA neurons. Reelin, known to control neuronal migration and positioning in the cerebral cortex, has recently been shown to be important for the normal positioning of DA neurons in the SNc. Indeed, DA neurons fail to migrate tangentially in the SNc of reeler mice and accumulate abnormally lateral to the VTA (Nishikawa et al., 2003).

Guidance of DA projections

The molecular signals that guide and confer target specificity of the different populations of midbrain DA neurons are poorly understood (Gates et al., 2004). So far, only a few molecules have been shown to play a role in promoting DA axon growth or guidance. The receptor EphB1 and its ligand ephrin-B2 have been shown to play a role in the guidance of DA projections of the SN (Yue et al., 1999). EphB1 and ephrin-B2

are expressed in a complementary pattern in midbrain DA neurons and their targets during the early postnatal period (Yue et al., 1999), when DA innervation occurs for the most part (Specht et al., 1977a,b). EphB1 is detected at high levels in the SN and not in the VTA and, complementarily, its ligand is highly expressed in the developing striatum, the nucleus accumbens and the olfactory tubercle (Yue et al., 1999). In addition, ephrin-B2 specifically inhibits neurite outgrowth and induces cell loss in SN, but not VTA neurons (Yue et al., 1999). Together, these results suggest that the interaction between EphB1 and ephrin-B2 may result in an inhibitory signal that restricts SN axons from innervating the ventro-medial striatum and promotes cell death of misconnected neurons (Yue et al., 1999). In addition, ephrin-B2 is upregulated after cocaine treatment in mice (Yue et al., 1999), suggesting that it could play a role in plasticity of the DA brain reward circuit and, consequently, may be involved in drug addiction mechanisms (Nestler et al., 1993).

Programmed cell death

Naturally occurring cell death in the midbrain DA system starts shortly before birth in rat and achieves an initial peak at postnatal day (P) 2 (Oo and Burke, 1997; Jackson-Lewis et al., 2000). A second peak of cell death occurs between P14 and P20. During these two periods, TH-immunoreactive cells die by apoptosis, as they display nuclear apoptotic chromatin clumps, TUNEL reactivity and caspase-3 immunoreactivity (Jackson-Lewis et al., 2000). Little is known about the specific programmed cell death mechanism in midbrain DA neurons. Of the three general pathways that mediate programmed cell death (the extrinsic, the intrinsic and the endoplasmic reticulum stress induced pathway), the intrinsic pathway seems likely to

operate. This pathway relies upon the availability of trophic factors that assures mitochondrial integrity (Fig. 3, Table 1). Members of the Bcl-2 family of proteins that act upstream to the mitochondria regulate the release of cytochrome c and the resulting activation of caspases. In mice overexpressing the anti-apoptotic protein Bcl-2 under the control of the TH promoter, the number of DA neurons is increased in the adult SN (Jackson-Lewis et al., 2000).

Target induced survival of DA neurons

The survival of SN neurons is regulated by interaction with their targets that provide trophic factors in limited quantities. Striatal preparations *in vitro* support the viability and differentiation of DA neurons. *In vivo,* mechanical or pharmacological (6-OHDA) disruption of striatal target and/or connectivity regulates the number of DA neurons in the SN. Interestingly, this alteration is inhibited in TH/Bcl2 animals. Several molecules have long been suspected to mediate the trophic action of the striatal target on SN neurons such as the glial cell line-derived neurotrophic factor (GDNF) family of ligands. GDNF was originally found to promote the survival of SN neurons *in vitro* (Lin et al., 1993). Moreover, GDNF is known to prevent the loss of DA neurons during programmed cell death and in lesion models of PD, and to affect their phenotype in diverse ways including increased neurite outgrowth, cell body size, and levels of TH and dopamine (Beck et al., 1995; Sauer et al., 1995; Tomac et al., 1995; Gash et al., 1996). The data on the function of GDNF in models of neurodegeneration is in contrast to the lack of phenotype in the brains of mice lacking GDNF. The other frequently studied neurotrophic factor of the GDNF family is neurturin (NTN). *In vivo,* NTN protects DA neurons from lesion-induced degeneration and increases the survival of

embryonic DA neurons transplanted into postnatal rats (Horger et al., 1998; Eggert et al., 1998; Rosenblad et al., 1999).

A few other trophic factors have been implicated in the survival and/or maturation of DA neurons. Cultured DA neurons are responsive to the serine protease thrombin, which changes the length, number of branches, and pattern of neurites (Debeir et al., 1998). Similarly, morphological changes could be induced in DA neurons by a member of the nerve growth factor protein family, brain-derived neurotrophic factor (BDNF). *In vitro*, BDNF has been implicated in the survival and differentiation of DA neurons. *In vivo*, mice overexpressing BDNF in dopamine-β-hydroxylase positive neurons display a 52% increase of TH-positive cells in the SNc (Alonso-Vanegas et al., 1999). The authors suggest that an increased anterograde transport of BDNF via the coerulo-nigral projection could rescue DA neurons from developmental cell death. Our analysis of mice lacking trkB, the high affinity receptor for BDNF and NT4, revealed no significant loss of DA neurons in the SN and VTA as judged by VMAT2- and TH-immunoreactivity, suggesting that the lack of trkB signalling does not affect per se the genesis and cell death of DA neurons (Vitalis et al., 2002 and T.V. unpublished data). Finally, studies of mice deficient for the TGFα gene revealed a 50% reduction in the number of DA neurons in the SN, but a normal complement of other midbrain DA neurons. This indicates that TGFα participates in the expansion or differentiation of this subtype of DA neuron (Blum, 1998).

Benefits of stem cell research

There are two principally different ways of using stem cells for grafting in PD: stem cells may be used to generate DA neurons, or to produce survival factors to protect both the grafted and the resident DA populations.

Dopaminergic neurons could be made from stem cells of four different sources: embryonic stem cells from the fertilized egg, neural stem cells from embryonic or adult brain, or from stem cells in other tissues. Such neurons can be generated in high yield from mouse embryonic stem cells *in vitro* (Lee et al., 2000). A dramatic improvement in this strategy resulting in an increase in the yield of cells expressing DA phenotype was obtained in mouse embryonic stem cells overexpressing Nurr1 (Kim et al., 2002). When the cells were grafted into the rat striatum, they survived and improved deficits resembling parkinsonian signs. Using a different approach, Kawasaki and co-workers (2002a,b) discovered that bone-marrow-derived stromal cell line was a potent inducer of neuronal differentiation. After co-culture, all cultures contained a significant yield of dopamine producing cells that showed substantial short-term survival after transplantation to the mouse striatum (Kawasaki et al., 2002a). Also when using primate embryonic stem cells, the stromal cell-derived inducing activity was able to generate neurons expressing several markers of DA neurons (Kawasaki et al., 2002b). Dopaminergic neurons have also been made from neural stem cells with various protocols *in vitro*. The generated cells survived transplantation to the striatum and ameliorated parkinsonian signs (Studer et al., 1998, 2000; Carvey et al., 2003; Wagner et al., 1999).

A high proportion of DA cells can be generated from mesenchymal stem cells taken from the adult bone marrow (Jiang et al., 2002). However, intravenous infusion of the mesenchymal stem cells did not give rise to any engraftment in the brain.

Finally, neural stem cells overexpressing neurotrophic factors are able to induce neuroprotective effects after grafting in animal models of PD. The GDNF family of neurotrophic factors is the family with the broadest and strongest trophic effects on midbrain DA neurons. Indeed neural stem cells overexpressing GDNF (Akerud et al., 2001) or persephin (Akerud et al., 2002) prevented the loss of DA neurons and the behavioral impairment of mice receiving intrastriatal injection of the PD inducing drug, 6-OHDA.

Conclusion

Recent data from molecular studies on the development of midbrain DA neurons point to several important genetic pathways. First, midbrain DA progenitors are specified by the combined action of Shh and FGF8. Then, transcription factors in early postmitotic midbrain neurons are organized at least in three genetic cascades. Nurr1 appears to be involved in the neurotransmitter synthesis, storage and uptake, but does not affect Lmx1b and Pitx3 expression. In contrast, Lmx1b-Pitx3 and En-1/En-2 do not seem to be required in dopamine synthesis, but the Lmx1b-Pitx3 and En-1/En-2 cascades are associated with aspects of DA neuron differentiation such as migration, survival and maintenance. Finally, members of the GDNF family are the best candidates to promote the survival of midbrain DA neurons and may constitute a very useful therapeutic strategy in the treatment of PD.

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Table

Table 1: Summary of factors involved in DA neuron development

Factor	Family	Member	Receptor associated	Aspect of DA identity regulated
Secreted factors				
	Sonic hedgehog	Shh		Specification
	Wnt	Wnt-1	Low-density lipoprotein receptor-related protein 6 (LPR6)	Proliferation
		Wnt-3a		Specification
		Wnt5a		
Neurotrophic factors	Neurotrophins	Brain-derived neurotrophic factor (BDNF)	Tyrosine kinase receptor Trk-B	Differentiation Target induced survival
		Neurotrophin-4		
	Fibroblast growth factor (FGF)	FGF8		Specification
	Transforming growth factor beta	TGFβ)		Specification Maintenance Survival
	Glial cell line-derived neurotrophic factors	GDNF	Tyrosine kinase receptor (c-Ret)	Target induced survival
		Neurturin (NTN)		Target induced survival
		Persephin		Target induced survival
Transcription factors				
Homeobox-containing genes	Bicoid-related homeodomain	Ptx3/Pitx3		Migration Maintenance Survival
	LIM-homeodomain	Lmx1b		Migration Maintenance Survival
	Paired-box	Pax-2		Specification
		Pax-5		Specification
		Pax-6		Axonal pathfinding
		Engrailed (En-1, En-2)		Survival
		Gbx2		Specification
		Otx-1, Otx2		Specification
Zinc finger		Gli-1		Specification
Superfamily of ligand activated transcription factor		Nuclear receptor Nurr-1		Specification Differentiation DA phenotype
Guidance molecule				
		Ephrin-B2	EphB1	Axonal pathfinding
Cell-adhesion molecules				
		Neural cell adhesion molecule L1/NgCAM		Migration
Proteoglycan	Chondroitine Sulfate	6B4		Migration
Extracellular matrix molecule				
		Reelin		Migration

Figures

Fig. 1. A. Schematic representation of the developing mouse central nervous system at E11.5. Mesencephalic dopaminergic (DA) neurons are generated in the immediate vicinity of two organizing centers, the floor plate (FP) in the ventral midline and the isthmus at the midbrain/hindbrain boundary. Morphogens such as Shh (purple) produced by the FP, FGF8 (orange) produced by the isthmus, and Wnt1 are represented. Expression of the homeobox-containing genes Pax-2 and Otx-2 that participate in DA specification is also indicated. III: cranial nerve III, MF: mesencephalic flexure; PC posterior commissure. B. Specification and transcriptional control of DA neuron development. Midbrain DA progenitors are specified by a combination of the morphogenetic factors Shh, FGF8 and wnt-1. At least three independent regulatory cascades have been characterized in early postmitotic, maturing and mature DA neurons. Nurr1 is involved in the DA neurotransmitter phenotype and in DA neuron survival and maintenance via c-ret, the high affinity receptor of GDNF. The En-1/En-2 cascade determines DA survival via α-synuclein. The lmx1b-Pitx3 regulatory cascade is involved in the maintenance of midbrain DA neurons, but the genetic targets are unknown.

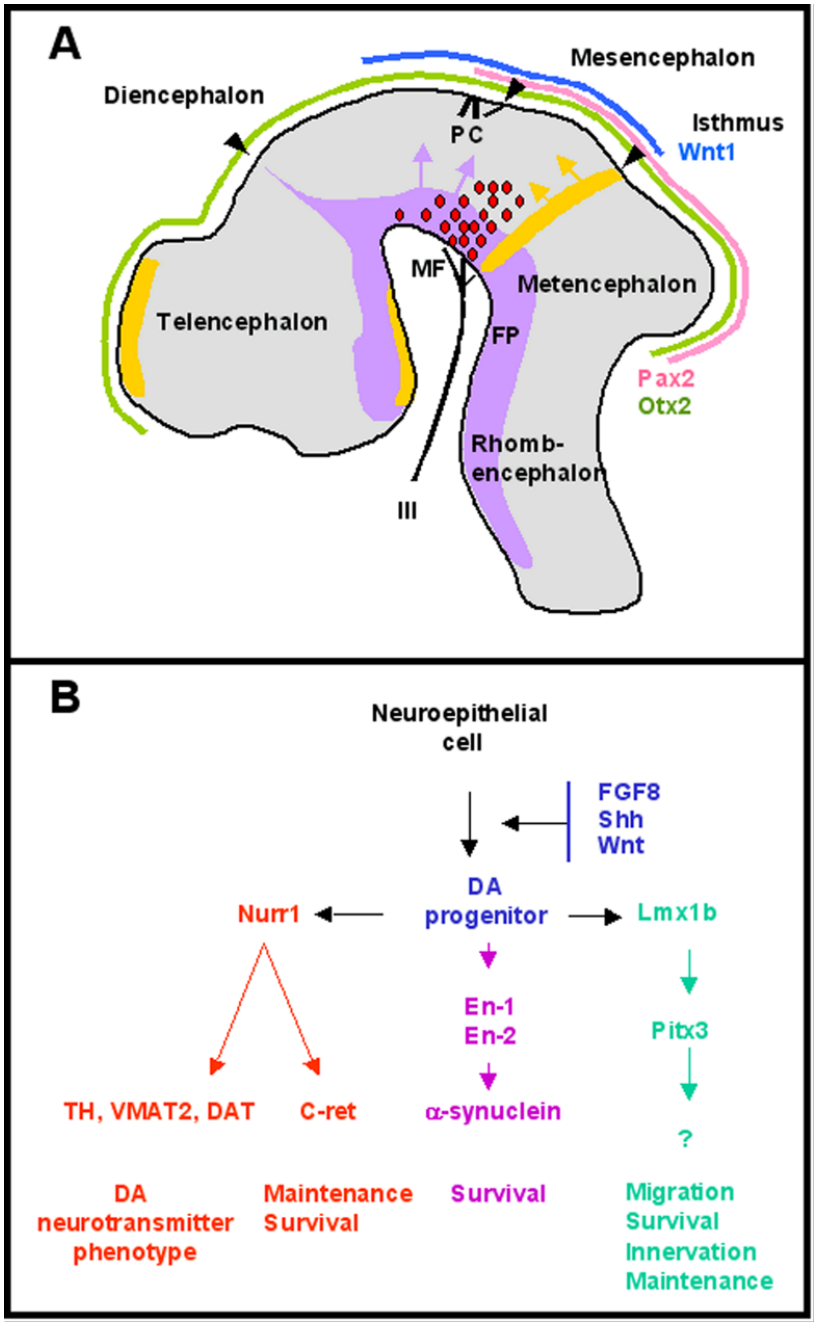


Fig. 2. Schematic representation of the mechanisms of cell migration during DA neuron development. In rat, early postmitotic DA neurons migrate radially along radial glial processes by E12 (1). Once they reach the floor plate, these cells migrate laterally along axons expressing the cell adhesion molecule L1 (2).

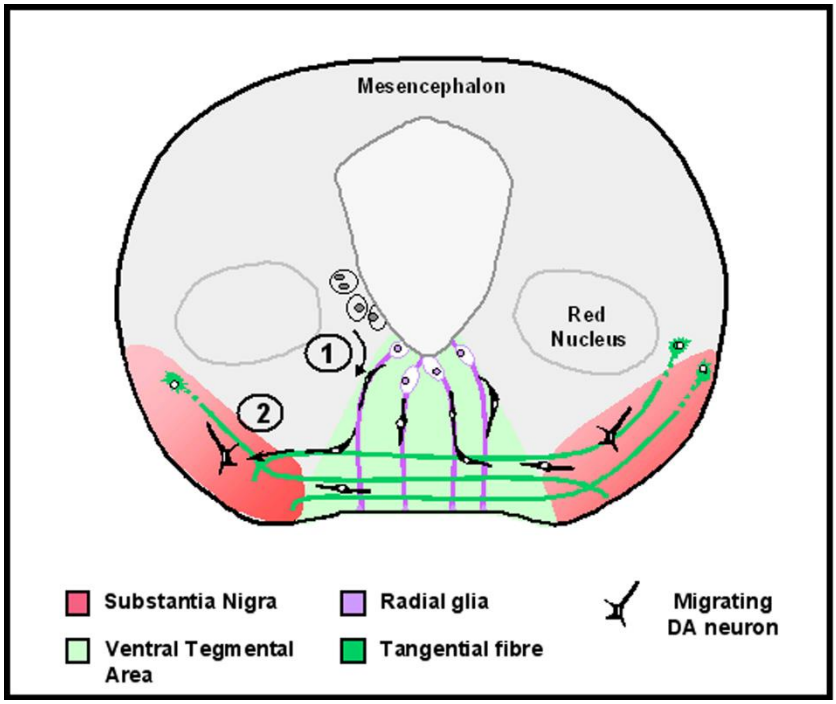
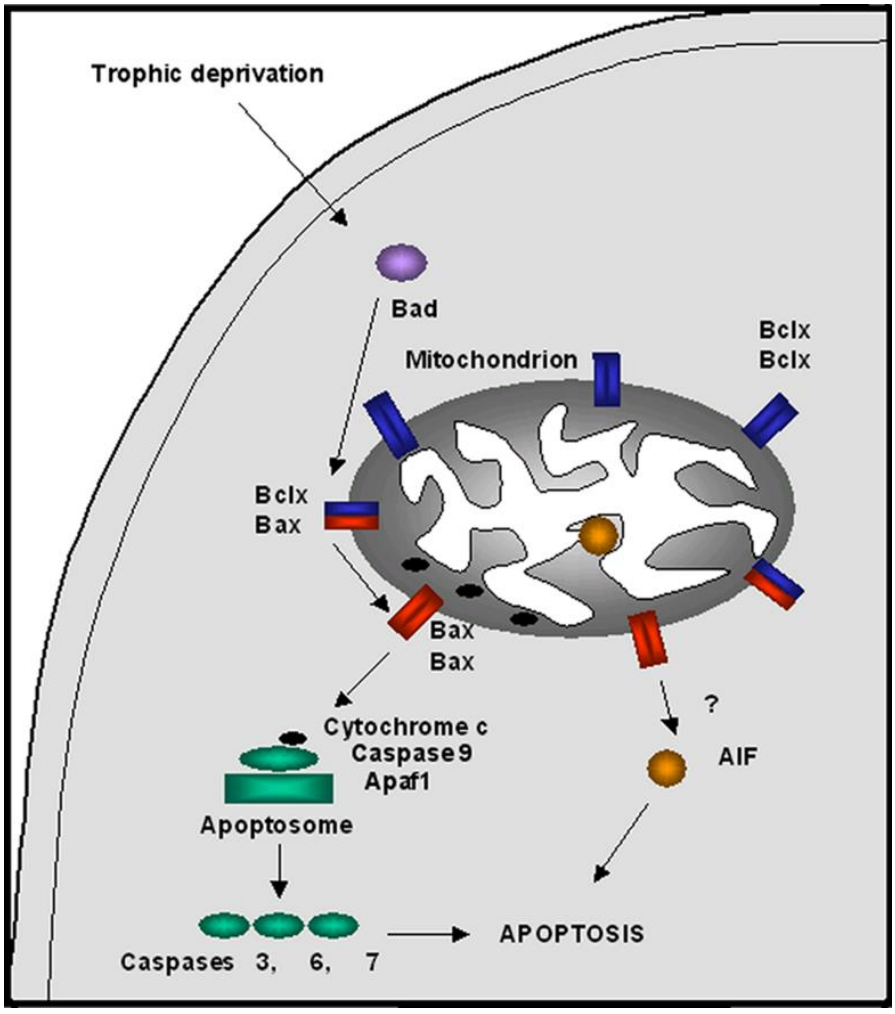


Fig. 3. Developmental cell death of DA neurons. The deprivation of specific trophic factors such as GDNF and BDNF induces the activation of pro-apoptotic molecules such as BAD. This activation disturbs the balance between anti-apoptotic (such as Bclx) and pro-apoptotic (such as Bax) molecules at the mitochondrial membrane leading to the release from the mitochondrial matrix to the cytoplasm of cytochrome c (cytc) and activated caspase 9. In the cytoplasm, Apaf1, cytc, and casp9 associate to form the apoptosome that induces apoptotic cell death by activating the terminal caspases, caspase-3, -6 and -7. Alternatively, the imbalance between pro- and anti-apoptotoc molecules could also lead to the release in the cytoplasm of AIF (apoptosis inducing factor) that induces apoptosis by a caspase-independent pathway.


Trophic deprivation
Bad
Mitochondrion
BclxBclx
BclxBax
BaxBax
Cytochrome c
Caspase9
Apaf1
Apoptosome
?
AIF
Caspases 3, 6, 7
APOPTOSIS