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Dissecting the role of Engrailed in adult dopaminergic neurons: Insights into Parkinson disease pathogenesis

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Abstract

The homeoprotein Engrailed (Engrailed-1/Engrailed-2, collectively En1/2) is not only a survival factor for mesencephalic dopaminergic (mDA) neurons during development, but continues to exert neuroprotective and physiological functions in adult mDA neurons. Loss of one En1 allele in the mouse leads to progressive demise of mDA neurons in the ventral midbrain starting from 6 weeks of age. These mice also develop Parkinson disease-like motor and non-motor symptoms. The characterization of En1 heterozygous mice have revealed striking parallels to central mechanisms of Parkinson disease pathogenesis, mainly related to mitochondrial dysfunction and retrograde degeneration. Thanks to the ability of homeoproteins to transduce cells, En1/2 proteins have also been used to protect mDA neurons in various experimental models of Parkinson disease. This neuroprotection is partly linked to the ability of En1/2 to regulate the translation of certain nuclear-encoded mitochondrial mRNAs for complex I subunits. Other transcription factors that govern mDA neuron development (e.g. Foxa1/2, Lmx1a/b, Nurr1, Otx2, Pitx3) also continue to function for the survival and maintenance of mDA neurons in the adult and act through partially overlapping but also diverse mechanisms.

Keywords

Dopaminergic neurons; Homeoproteins; Parkinson disease; Engrailed; Mitochondria; Substantia nigra pars compacta; Retrograde degeneration

1 Introduction

Mesencephalic dopaminergic (mDA) neurons in the ventral midbrain are the main source of dopamine (DA) in the adult brain. Dysfunctions in the signaling of this neurotransmitter is involved in several neurological and psychiatric disorders [1–3]. In recent years, tremendous progress has been made in dissecting the genetic networks and signaling pathways that govern various steps of mDA neuron development such as patterning, induction and specification of mDA progenitors, and their subsequent differentiation into mature mDA neurons. These studies mainly performed using transgenic mouse models have revealed that

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mDA neuron ontogenesis is controlled by the concerted action of several transcription factors including Engrailed-1/Engrailed-2 (collectively Engrailed or *En1/2*), *Foxa1/2*, *Lmx1a/b*, *Nurr1*, *Otx2* and *Pitx3*, and growth factors or morphogens such as *Shh*, *Fgf8*, *Tgf-β* and *Wnt1* [4–6]. It was shown for instance that *En1/2* alleles are critically required for the survival and maintenance of mature mDA neurons during late embryonic life in a dose-dependent manner [7–10]. Other transcription factors such as *Nurr1* and *Pitx3* play key roles in the acquisition of a mature mDA neuron identity during development by regulating the expression of several mDA neuron-specific markers such as tyrosine hydroxylase (TH) and dopamine transporter (DAT) [5, 6].

Interestingly, a number of recent studies have shown that several transcription factors, which control mDA neuron development, display continued expression in adult mDA neurons and are required for the maintenance of these neurons throughout life [11, 12]. These findings are particularly interesting in the context of Parkinson disease (PD), since possible genetic links between some of these transcription factors (e.g. *EN1/2*, *NURR1*, *PITX3*, *LMX1A/B*) and PD have been reported [13–17], although these findings await further confirmations. Deciphering the role played by these transcription factors in mDA neurons during development and in the adult, and elucidating the underlying mechanisms are an emerging field in PD research.

This review is mainly focused on the homeoprotein Engrailed, which belongs to the class of DNA binding homeodomain containing transcription factors. The expression of *En1/2* during development starts early at embryonic day E8 in a patch of cells in the anterior neuroepithelium in the midbrain hindbrain boundary region. The extension of brain expression of *En1/2* changes during the course of development and then confines to midbrain and hindbrain structures in the adulthood [18]. In the midbrain, *En1/2* expression in the adult becomes highly restricted to mDA neurons of the substantia nigra pars compacta (SNpc), which preferentially degenerate in PD, and of the ventral tegmental area (VTA) [7, 8]. Interestingly, recent work, using genetic and toxicological mouse models, support the idea that *EN1/2* might be in the PD pathway [19–22]. We review here the lessons emerging from these studies, which bring important insights into mDA neuron physiology, our understanding of PD pathogenesis and which open new perspectives for PD therapeutics.

2 Engrailed and PD connection

2.1 Hallmarks of PD

The main hallmark of PD, which represents the second most common neurodegenerative disorder after Alzheimer's disease, is the slow and progressive loss of mDA neurons of the SNpc [1–3]. These neurons project to the dorsal striatum and form the nigrostriatal pathway, involved in the control of voluntary movements. The massive loss of these neurons ultimately results in severe striatal DA deficiency, which leads to classical PD-associated motor symptoms such as tremor, rigidity, bradykinesia and postural instability. Another hallmark of PD is the presence of intraneuronal inclusions mainly containing α -synuclein protein aggregates within the cell body (Lewy bodies) or processes (Lewy neurites). Age is the major risk factor for the development of PD [23], and the prevalence and incidence greatly increase after the age of 80 years. Exposure to environmental factors (i.e. pesticides)

can also increase the risk to develop the disease [3]. Diagnosis of PD occurs with the onset of motor symptoms, but this can be preceded by a pre-motor or prodromal phase of 20 years or more [3]. It has been assumed for long time that the first motor symptoms appear when more than 80% of striatal DA is lost [1]. However, a reassessment of all available data suggests that the loss of SNpc mDA neurons remains asymptomatic until 30% of mDA neuron cell bodies and 50-60% of axonal terminals are lost [24]. In any case, adaptive changes in DA terminals and postsynaptic striatal neurons appear to compensate for significant losses of striatal DA to preserve motor behavior. It was recently shown that toxigenetic ablation of mDA neurons in the mouse following targeted expression of the diphtheria toxin A gene during development results in a depletion of more than 95% of striatal DA and this does not lead to any motor symptoms in young-adult or aged mutant mice, suggesting that the ability to compensate for severe DA deficiency might be higher in the mouse than in humans [25]. Thus, early diagnosis can be instrumental for disease management but this still remains a major hurdle. Currently available treatments provide only symptomatic relief and do not modify the course of the disease [26].

Genetic studies in the past 15 years have identified a number of genetic mutations (e.g. gene duplications/triplications or missense mutations) in certain familial autosomal dominant or recessive monogenic forms of PD (i.e. SNCA, Parkin, PINK1, LRRK2, DJ-1, VPS35, ATP13A2, GBA) [27, 28]. Recessively inherited Parkinsonism is frequently associated with early disease onset (before the age of 40 years). Although the majority (>90%) of PD forms are sporadic, the phenotypes of familial and sporadic forms are very similar, implying that they might arise from common underlying mechanisms [3, 29]. The study of the function of PD-linked genes has been instrumental to advance our understanding of the cellular processes involved in PD pathogenesis as discussed below. However, most of the genetically engineered mouse models generated through changes in PD-linked gene expression do not exhibit the cardinal feature of progressive loss of mDA neurons in the SNpc [30, 31]. To this respect, genetically engineered mice, in which expression of transcription factor genes controlling mDA neuron development are altered, have proven very useful animal models for PD research [11].

2.2 Progressive loss of mDA neurons in *En1*^{+/-} mice

Previous loss of function studies demonstrated that *En1/2* protect mature mDA neurons during late embryonic development against caspase-3-mediated apoptotic cell death in a dose-dependent manner [7–10]. Subsequently, several studies using various mouse models with targeted disruption of *En1/2* alleles showed that *En1/2* continues to be required for the survival of adult mDA neurons. The effects of a complete loss of *En1* on adult mDA neuron survival could not be analyzed due to neonatal lethality of *En1*^{-/-} mutants (OF1 background) [32]. However, the phenotype of mice heterozygous for *En1* (*En1*^{+/-}) has now been extensively characterized by several groups [19, 21, 22, 33, 34]. These mice present a normal number of mDA neurons in the SNpc until the age of 6 weeks after birth. After this age, these neurons start to die progressively and their number decreases by about 40% (as compared to wild-type mice) at 48 weeks of age when their number is stabilized. *En1/2* gene dosage effect on survival was also seen in *En1*^{+/-}; *En2*^{-/-} mice (C57BL/6 background) which present a massive loss of mDA neurons in the SNpc of young adult mice. It was

shown that *En1/2* survival activity on mDA neurons in these mice could be mediated through both, the activation of Erk1/2 MAPK survival pathway and suppression of the pro-apoptotic activity of the pro-neurotrophin receptor p75NTR [10]. Finally, a recent study also analyzed *En1*^{-/-} mice, which are viable on a C57BL/6 background, and reported a much more rapid and pronounced postnatal loss of mDA neurons [35]. All these observations support the idea that *En1/2* continues to be required for the survival and/or maintenance of a subset of mDA neurons during adulthood.

It is noteworthy that VTA mDA neurons, located in the vicinity of SNpc, are relatively spared in *En1*^{+/-} mice (20% loss at 48 weeks) as is the case in human PD [36]. These neurons project to the nucleus accumbens, the amygdala, the hippocampus and the prefrontal cortex to form the mesolimbic and mesocortical pathways, involved in motivation, reward, addiction, cognition and memory. The molecular determinants of the selective vulnerability of mDA SNpc neurons as compared to the VTA are still not known. Some of these differences may arise from the fact that SNpc mDA neurons express elevated levels of a highly active glycosylated form of DAT (glyco-DAT) and inwardly rectifying potassium channel (GIRK2), whereas mDA neurons in the VTA express more of the calcium binding protein calbindin D28K (CALB1) [36]. More recent single cell gene expression profiling indicates that there might be a much greater heterogeneity in mDA neurons within the SNpc or VTA [37]. For instance, *En1* expression appears to be higher in mDA neurons located in the lateral ventral part of the SNpc [37], which hosts neurons that preferentially die in *En1*^{+/-} mice; mDA neurons in the ventral tier of the SNpc are also the most vulnerable in human PD.

Loss of SNpc mDA neurons in *En1*^{+/-} mice leads to decreased striatal DA. These mice develop PD-like motor symptoms such as reduced locomotor activity (distance travelled, rearing), increased amphetamine-sensitization, and defective motor coordination (rotarod). These mice also present some non-motor phenotypic alterations, such as depressive-like behavior (forced swimming test), anhedonic-like behavior (saccharine preference) and poor social interaction indicating that the moderate loss of mDA neurons in the VTA could affect the mesolimbic system in *En1*^{+/-} mice [19]. *En1* haplodeficient mice thus represent a valuable animal model to gain insight into the mechanisms leading to mDA neuron degeneration in PD.

3 Insights from *En1*^{+/-} mice into PD pathogenesis

The molecular and cellular mechanisms involved in PD pathogenesis are still not entirely elucidated. However, the picture emerging from a number of studies is that PD pathogenesis involves dysregulation of several interconnected cellular processes and molecular pathways [1, 3, 29]. A major pathogenic pathway, which received particular attention, points towards mitochondrial dysfunction [38]. This is not only related to mitochondrial energy production, but also to many aspects of mitochondrial dynamics such as fusion-fission, trafficking, and quality control [39, 40]. Other pathogenic culprits include oxidative stress, accumulation and/or aggregation of misfolded proteins, defects in the ubiquitin-proteasome system, impairment in the lysosome-autophagy pathway, altered vesicular-organelle trafficking, defective synaptic function, neuro-inflammation, and prion-like cell-to-cell transmission of

α -synuclein aggregates [1, 3, 29]. The major players contributing to PD pathogenesis are shown in Figure 1. Recent studies have also suggested that nucleolar stress, defective DNA damage/repair pathways and associated chromatin modifications might also play an important role in many neurodegenerative diseases including PD [41–45]. We briefly review here the contribution of *En1*^{+/-} mouse model for dissecting the mechanisms of PD pathogenesis, particularly those related to mitochondrial activity and retrograde degeneration of mDA neurons involving synaptic dysfunction and autophagy.

3.1 Mitochondrial dysfunction and its link to PD

Historically, the impairment of complex I of the mitochondrial respiratory chain has long been considered as the key culprit in PD pathogenesis [38]. This concept was based on several findings: i) complex I inhibitors such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) or rotenone can cause PD-like symptoms in rodents, non-human primates or even humans after accidental intoxication, ii) complex I activity was found to be reduced in postmortem PD brain tissues, iii) mutations in complex I subunits such as the nuclear-encoded NDUFV2 were associated with PD, iv) large-scale deletions in mitochondrial DNA were reported specifically in the SNpc of patients with sporadic PD, and v) several proteins encoded by genes mutated in PD (i.e. *PINK1*, *Parkin*, *DJ-1*, *LRRK2* or *SNCA*) were found to be associated with mitochondria [46]. The complex I hypothesis is quite attractive, as mDA neurons are the highest energy consuming cells in the brain and rely on a very active mitochondrial respiratory chain/oxidative phosphorylation (OxPhos) system for ATP production [47]. This is intricately coupled to elevated ROS (reactive oxygen species) generation, which, in turn, causes oxidative stress and cellular damage [39, 40, 46].

The idea that a failure to sustain the high-energetic demand of mDA neurons can be crucial in PD pathogenesis was demonstrated in the MitoPark mouse model, generated by targeted disruption of the mitochondrial transcription factor Tfam. These mice present respiratory chain deficiencies and develop several features of PD, including progressive degeneration of the nigrostriatal system [48]. These mice also present behavioral phenotypes, associated to striatal dysfunction, which precede the loss of mDA neurons in the SNpc [49], suggesting retrograde degeneration (see below). The hypothesis that bioenergetics defects might contribute to PD pathogenesis was also supported by the strong association of PD with genes encoding OxPhos subunits and enzymes of the glucose metabolism. These genes are all regulated by the transcription factor PGC-1 α , a positive regulator of mitochondrial biogenesis and cellular antioxidant responses [50]. Interestingly the PD-linked gene *Parkin* appears to be a positive regulator of PGC-1 α by constantly degrading PARIS (Parkin interacting substrate), a negative regulator of PGC-1 α . Mutations in *Parkin* can increase PARIS protein levels leading to decreased PGC-1 α and associated energetic deficits [51]. Finally, a recent study has shown that elevated mitochondrial bioenergetics and axonal arborization size could be key contributors to the vulnerability of SNpc mDA neurons [52].

The possible link between *En1/2* and mitochondria was recognized while studying the role of *Engrailed* in axon guidance. It is important to recall that many homeoproteins, including *Engrailed*, are endowed with the ability to be secreted and internalized by live cells. The sequences which allow *En1/2* to transduce cells have been characterized and are located

within the DNA binding homeodomain [53, 54]. Physiological roles of homeoprotein non-cell autonomous activity of homeoproteins have now been found in several system; in axon guidance for Engrailed, in the critical period and cortical plasticity for Otx2, and in eye development for Pax6 [18, 55–60]. Moreover, in addition to being a transcription factor, Engrailed can also regulate translation, thanks to its ability to bind the translation initiation factor eIF4E, another common feature of many homeoproteins [61, 62]. In the context of Engrailed and axon guidance, it was shown that Engrailed protein transduction can guide retinal axons and that Engrailed activity is dependent on its ability to function as a translation regulator through the mTOR pathway [55, 63]. The search for Engrailed translation targets showed that En1/2 protein transduction can stimulate the translation of nuclear-encoded mitochondrial mRNAs for certain complex I subunits (e.g. Ndufs1/Ndufs3), leading to increased ATP production in retinal growth cones or elevated complex I activity in midbrain synaptoneurosome [20, 63]. In line with these observations, the level of Ndufs1 was decreased in mDA neurons of *En1*^{+/-} mice which may reflect a bioenergetics problem underlying mDA neuron degeneration in these mice [20]. Finally, another En1/2 translation target, identified in *Xenopus* retinal axons, is Lamin B2, a major constituent of the nuclear envelope. Lamin B2 translation in axons regulates mitochondrial size, mitochondrial membrane potential and supports axon survival [64]. Mitochondria are critically required for long-term axonal survival and maintenance [65]; En1/2 might thus play an important role to sustain mitochondrial activity for axon maintenance throughout adulthood.

The importance of translation regulation for mitochondrial function was recently underscored in a study showing that the PD-linked genes *PINK1* and *Parkin* control localized translation of nuclear-encoded respiratory chain component mRNAs on the mitochondrial outer membrane [66]. Some of the translationally repressed mRNAs, which localize on mitochondria in a PINK1/Tom20-dependent manner, are activated by PINK1/*parkin* through displacement of the attached repressors and enhanced binding of translational activators such as eIF4G [66]. More generally, it was recently hypothesized that deregulation of translation control might be a crucial factor in PD pathogenesis, which might have so far been overlooked [67]. The translation initiation factor eIF4G1 might be in the PD pathway [68, 69] possibly also through its ability to reprogram translation under stress conditions [70].

Mitochondria also appear to be targets of Nurr1, another developmental transcription factor critically required for mDA neuron maintenance in the adult. Indeed, several nuclear-encoded mitochondrial genes were identified as potential Nurr1 transcriptional targets [71]. Finally, gene expression profiling in the MN9D dopaminergic cell line also identified nuclear-encoded mitochondrial subunits of the respiratory chain as potential Lmx1a transcriptional targets, suggesting a possible link also between Lmx1a and mitochondria [72].

Although the complex I hypothesis has been central for PD pathogenesis for almost two decades, recent work suggest that other mitochondrial alterations could also be critical in PD pathogenesis [46]. Indeed, PD-linked genes such as *PINK1* and *Parkin* that localize to mitochondria also critically regulate mitochondrial dynamics (e.g. control of mitochondrial fusion/fission, or mitochondrial trafficking and axonal transport, as well as mitochondrial

quality control). The term ‘mitochondrial quality control’ encompasses the degradation of damaged mitochondrial proteins via autophagy, the export of misfolded and/or aggregated proteins through mitochondria-derived vesicles towards lysosomes/peroxisomes for degradation and the elimination of damaged mitochondria by mitophagy. Alterations in such processes may ultimately lead to energetic deficits [46, 73–75]. It will be interesting to examine whether mDA neurons in *En1*^{+/-} mice present any defects in mitophagy since the autophagy/mitophagy marker LC3 was decreased in mDA neurons in these mice (see below) [21].

3.2 Retrograde degeneration of mDA neurons

It was proposed several years ago that mDA neuron death in PD could be a « dying-back » degeneration process, considering that the DA transporter DAT was particularly enriched in striatal terminals rather than on SNpc mDA neuron cell bodies [76]. A recent assessment of available data further supports the idea that the loss of striatal dopaminergic axon terminals can indeed be an early and dominant feature of PD, as the extent of loss of striatal dopaminergic markers largely exceeds that of SNpc mDA neuron loss at the time of motor symptom onset [24, 77, 78]. The synaptic and axonal pathologies observed with the PD-linked genes SNCA and LRRK2 also suggest retrograde degeneration of mDA neurons in cellular or mouse models of PD. Several studies indicate that disease-related effects of α -synuclein first occur at pre-synaptic terminals where the protein is predominantly localized. It was shown for instance that exogenous α -synuclein fibrils can induce intraneuronal aggregates of endogenous α -synuclein, which initially form at pre-synaptic terminals and then propagate retrogradely to the neuronal soma [79]. Accumulation of pathologic α -synuclein leads to a selective decrease in synaptic proteins and reduced DA release, progressive alterations in neuronal excitability and connectivity and eventually to cell death. Similarly, the pathogenesis resulting from mutations in *LRRK2*, the most common genetic form of PD, may also initially involve axonal pathology. Transgenic mice expressing the disease-causing human *LRRK2* mutation (R1441G) exhibit fragmented TH-positive axons associated with axonal spheroids and display dystrophic neurites [80], although there is no loss of mDA neurons in this model. As mentioned above, impaired mitochondrial dynamics can greatly contribute to PD pathogenesis. It was shown that conditional knockout of the *mitofusin 2* (*Mfn2*) gene, encoding an essential factor for mitochondrial fusion, results in loss of dopaminergic terminals in the striatum, which occurs much earlier than the onset of mDA neuron loss [81]. Accumulation of fragmented mitochondria as well as reduced mitochondrial mass and transport resulting from *Mfn2* deficiency may ultimately contribute to neuronal loss. Of note, mDA neuron death in the classical MPTP neurotoxin model of PD might as well initially involve degeneration of distal axons [78, 82]. In conclusion, all these observations point to a retrograde mode of mDA neuron degeneration.

The phenotype of *En1* heterozygous mice strongly pleads for a retrograde degeneration of the nigrostriatal system. These mice display early signs of degeneration of mDA axons terminals in the striatum, which precede the loss of mDA neuron cell bodies [21]. Dopaminergic terminals become dystrophic and swollen, contain autophagic vacuoles and present deficits in DA release and uptake. Individual axons in the nigrostriatal pathway of *En1*^{+/-} mice undergo fragmentation, supporting the idea that axonal transport failure might

be an early feature of PD [83]. The nigral dopaminergic cell bodies also exhibit signs of decreased autophagy accompanied by an increase in mTOR activity and a decrease of the autophagic marker LC3B [21]. Impairment of autophagy is considered as an important culprit in PD pathogenesis [84–86]. Its relevance was recently assessed in a mouse model generated by the conditional deletion of the autophagy-related gene *Atg7*, which recapitulates many pathologic features of PD, including age-related loss of mDA neurons [87]. In conclusion, *En1*^{+/-} mouse represents a valuable model for PD and Table 1 recapitulates the phenotypic alterations resulting from *En1* haploinsufficiency.

Degeneration of mDA neurons in a retrograde fashion has also been suggested from the study of conditional knockout mice for *Lmx1a*, in which mDA neurons are progressively lost both in the SNpc and the VTA [17]. Prior to the onset of mDA cell body loss, these mice present abnormally large striatal nerve terminals that are filled with autophagic and lysosomal vesicles, very much like *En1*^{+/-} mice. In these mice, alteration of the autophagy/lysosomal pathway could be a consequence of increased mTOR activity in mDA neurons as in *En1*^{+/-} mice [21]. Interestingly, rapamycin treatment normalizes the phenotypic alterations resulting from targeted *Lmx1a* disruption [17]. Finally, conditional inducible deletion of *Nurr1* in mDA neurons in mice (5 weeks-old) results in a progressive pathology, associated with loss or reduced striatal DA content, impaired motor behavior, as well as dystrophic axons and fragmented dendrites containing varicosities [71], although no major loss of mDA neurons was reported in these mice.

4 Engrailed as a therapeutic protein for PD

As already mentioned above, several homeoproteins have the ability to transduce cells [53, 54] and this feature makes them particularly attractive for therapeutic purposes. Taking advantage of this property, it was demonstrated that En1/2 protein transduction provides efficient neuroprotection in various contexts. It was first shown that loss of mDA neurons in *En1*^{+/-} mice can be halted by infusing recombinant En1/2 proteins (En1 and En2 are biochemically equivalent in the adult midbrain) in the SNpc [19]. More importantly, En1/2 protein transduction-mediated protection of mDA neurons was reported in several experimental models of PD, *in vitro* and *in vivo*, including the MPTP, rotenone, 6-OHDA (6-hydroxydopamine) and mutated α -synuclein (A30P) models [20]. Intriguingly, unilateral Engrailed infusion in naive mice increases striatal DA content on the ipsilateral (injected) side. This leads to an amphetamine-induced turning behavior contralateral to the side of infusion, indicating an activation of the nigrostriatal pathway following Engrailed infusion in the SNpc [20]. This observation is interesting from a therapeutic point of view since En1/2 is able not only to protect mDA neurons in several PD models, but also to enhance the physiological activity of mDA neurons on a naive background. From a mechanistic point of view, it was shown that Engrailed-mediated protection of mDA neurons against MPTP was partly linked to its ability to up-regulate the translation of certain nuclear-encoded mitochondrial complex I subunits (e.g. Ndufs1/Ndufs3), thus enhancing complex I activity and ATP synthesis [20, 63].

In the context of homeoproteins and neuroprotection, it is also noteworthy to describe some of the work related to Otx2, an important regulator of mDA neuron subtype identity during

development [88]. In the adult, *Otx2* continues to be expressed in a subset of mDA neurons in the central and medio-lateral area of the VTA [89]. Conditional knockout of *Otx2* in adult mice results in selective loss of the axonal projections from VTA mDA neurons [90, 91]. *Otx2* acts as a negative regulator of DAT and an inverse correlation between *Otx2* expression and glyco-DAT (highly active form of DAT) levels in mDA neurons has been reported [89]. It was shown that *Otx2* gain of function in SNpc mDA neurons reduces glyco-DAT levels, and protects them from MPTP toxicity [89]. Finally, it was reported that forced expression of *Otx2* in mDA neurons in the SNpc of *En1*^{+/-} mice also prevents the progressive loss of mDA neurons caused by *En1* haploinsufficiency [33]. It is likely that *Otx2* and *En1/2* share some common neuroprotective mechanisms. Also, *Otx2* expression in the VTA mDA neurons might explain that these neurons are relatively spared in *En1*^{+/-} mice. *Otx2* protein transduction could thus also be of potential therapeutic interest for neuroprotection in PD. It is noteworthy that *Otx2* protein transduction has previously been reported to save retinal ganglion cells (RGCs) from NMDA (N-methyl-D-aspartate)-induced death in a mouse model of glaucoma [92].

Finally, the transcription factors *Nurr1*, *Foxa1/2* and *Pitx3* might also engage other mechanisms for neuroprotection of adult mDA neurons. For instance, *Nurr1/Pitx3* are involved in transcriptional regulation of the GDNF-RET-BDNF axis for neurotrophic support [93–95]. *Nurr1* was also reported to be a downstream target of CREB for survival signaling [96]. Finally, *Nurr1/Foxa2* might also act in microglia and astrocytes to decrease pro-inflammatory cytokine production and to increase the secretion of neurotrophic factors [97, 98]. Figure 2 provides a summary of mechanisms and signaling pathways engaged by transcription factors to confer dopaminergic neuroprotection in the adult brain.

5 Concluding remarks

The study of genetically engineered mice with altered expression of transcription factors involved in mDA neuron development has provided valuable insights in our understanding of adult mDA neuron maintenance and PD pathogenesis, mainly related to mitochondria, autophagy and retrograde degeneration. In view of the phenotype of *En1*^{+/-} mice, it will be interesting to further explore possible connections between *EN1/2* and PD. Recent studies have revealed that these transcription factors might be linked to several neuroprotective mechanisms and survival pathways in adult mDA neurons. It is interesting to point out that many interconnections between *En1/2*, *Nurr1*, *Lmx1a/b*, *Pitx3*, *Foxa1/2*, GDNF/Ret, BDNF, PGC-1 α , nuclear-encoded mitochondrial gene expression, pro-inflammatory cytokines and PD-linked genes such as α -synuclein, *Pink1* or *Parkin* have been recognized. It will be interesting to examine whether *En1/2* could regulate any PD-linked genes. The dissection of the molecular mechanisms underlying neuroprotection conferred by transcription factors could help identifying novel therapeutic targets for PD. Thanks to their ability to transduce cells, *En1/2* and *Otx2* have also been used directly to protect mDA neurons in various experimental PD models [19, 20, 92]. It will be interesting to further evaluate the therapeutic potential of these transcription factors in non-human primate models of PD. More generally, protein transduction technology using cell penetrating peptides can offer an attractive alternative to viral vector-based gene delivery for therapeutic purposes [99–101].

The phenotypes of several genetically engineered mouse models of PD strongly revive the idea that mDA neurons may degenerate in a retrograde fashion [78], and this could be a common feature of many neurodegenerative disorders [102]. It should be pointed out that most of the strategies tested so far to achieve neuroprotection in PD were targeted to save the neuronal cell bodies, and much less efforts were devoted to prevent the retrograde degeneration process, which might actually precede the loss of neuronal somas. The mechanisms underlying death of neuronal cell bodies and those involved in axon degeneration might be quite different [78]. In this regard, it is noteworthy that overexpression of a constitutively active form of Akt was shown to strongly suppress retrograde degeneration of dopaminergic axons in a neurotoxin (6-OHDA) rodent model of injury, and this protection was linked to mTOR signalling and inhibition of macroautophagy [103]. The phenotypic correction of retrograde degeneration in Lmx1a conditional knockout mice by rapamycin is also quite compelling [17]. It will be interesting to examine in future studies if En1/2 or Otx2 protein transduction can prevent retrograde degeneration of mDA neurons in experimental PD models.

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Abbreviations

En1/2	Engrailed-1/Engrailed-2
DA	dopamine
DAT	dopamine transporter
mDA	mesencephalic dopaminergic
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
6-OHDA	6-hydroxydopamine
PD	Parkinson disease
SNpc	substantia nigra pars compacta
TH	tyrosine hydroxylase
VTA	ventral tegmental area

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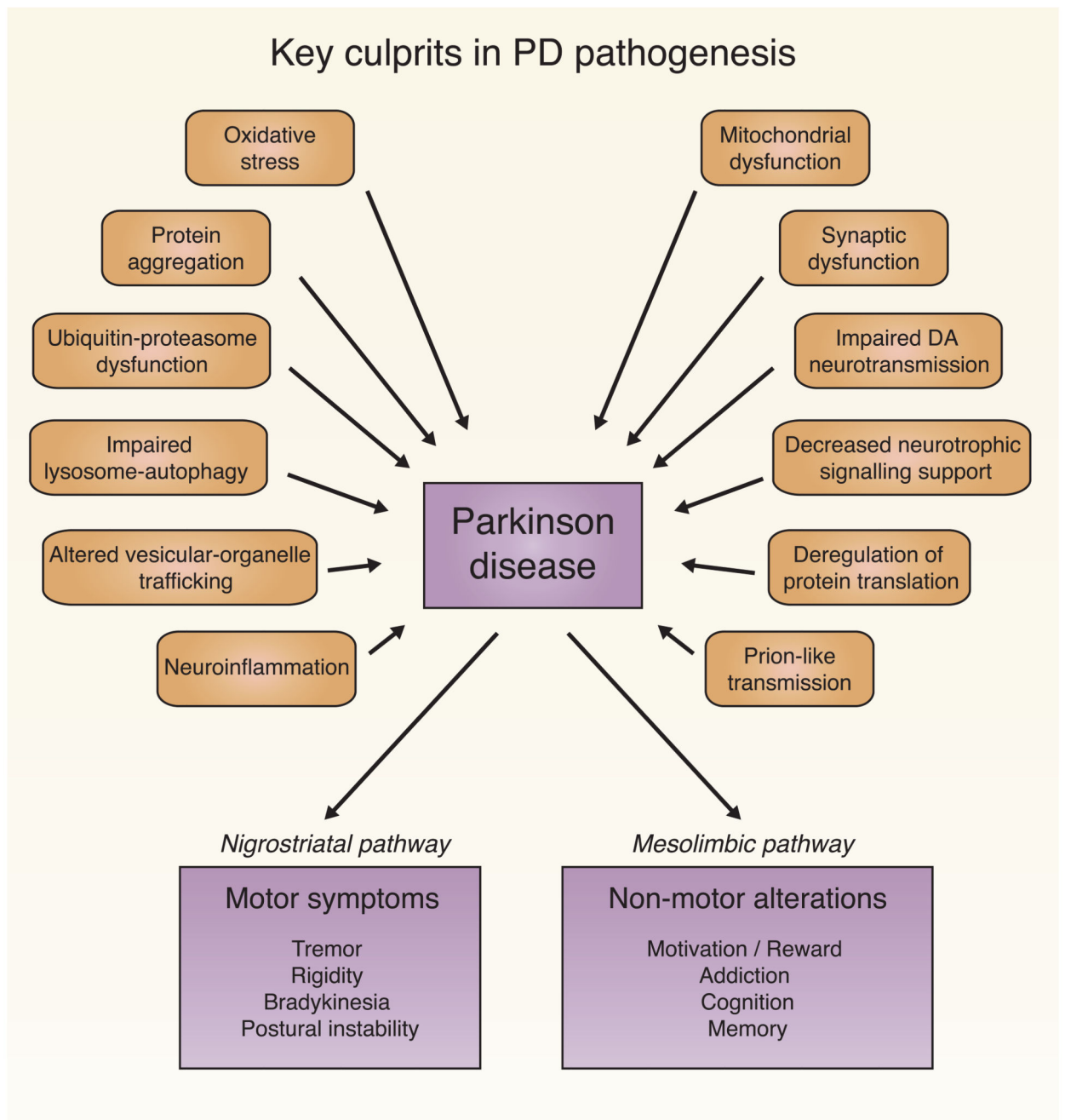


Fig. 1. Alterations in various cellular processes and molecular pathways contribute to PD development and associated motor and non-motor symptoms.

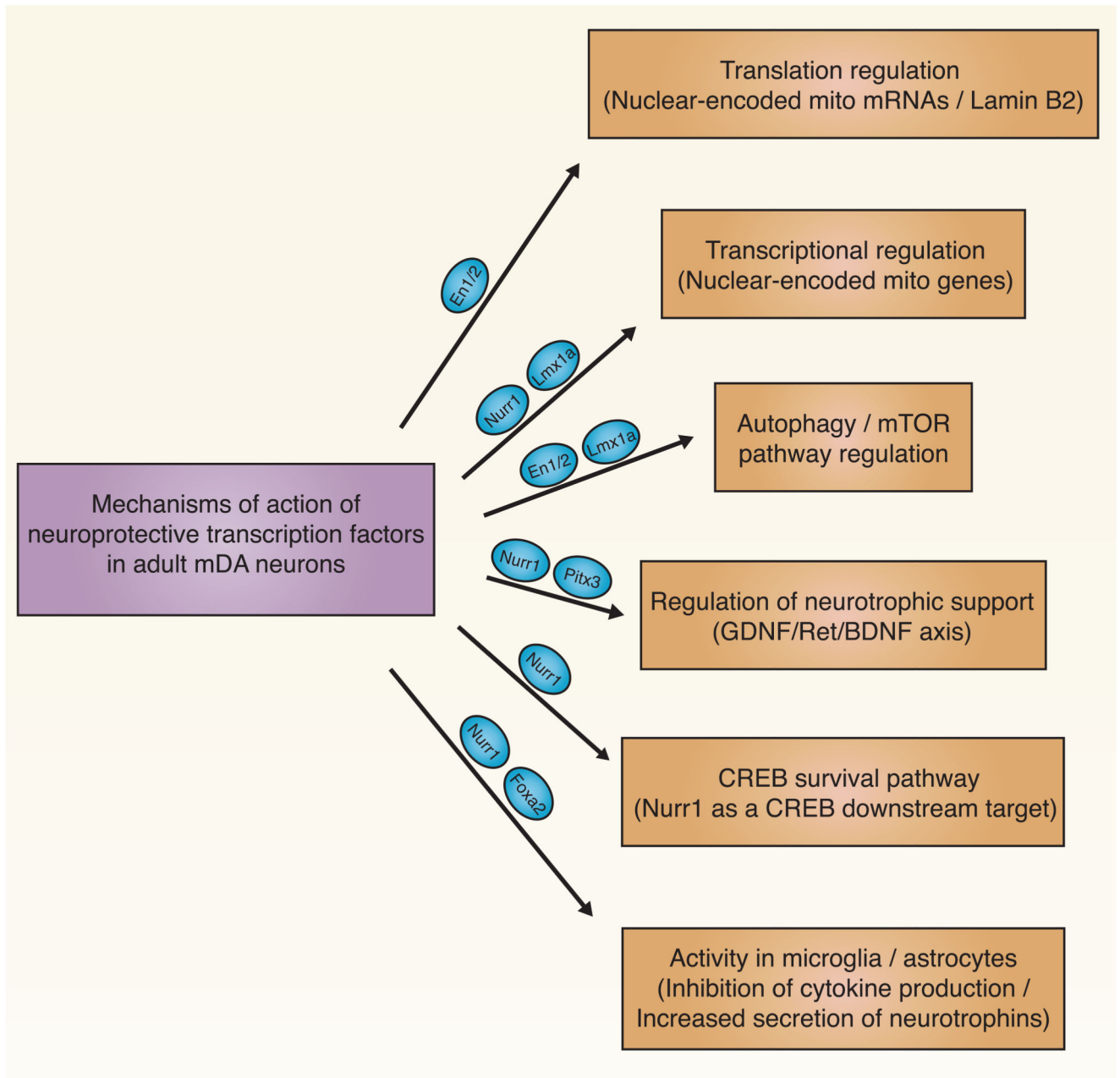


Fig. 2. Mechanisms and signaling pathways engaged by transcription factors to confer dopaminergic neuroprotection in the adult brain.

Table 1Phenotypic alterations in *En1*^{+/-} mice.

At the cellular and molecular level
Preferential loss of SNpc mDA neurons
Impaired autophagy
Increased mTOR activation
Axonal fragmentation
Defective DA release and uptake
Decreased nuclear-encoded mitochondrial proteins
At the motor and non-motor behavioural level
Reduced locomotor activity (distance travelled, rearing)
Increased amphetamine-sensitization
Defective motor coordination (rotarod)
Depressive-like behaviour (forced swimming test)
Anhedonic-like behaviour (saccharine preference)
Poor social interaction
