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Dopaminergic Differentiation Potential of Neural Precursor Cells Derived from Embryonic Stem Cells

Gilda Guerrero-Flores and Luis Covarrubias

*Department of Developmental Genetics and Molecular Physiology,
Instituto de Biotecnología. Universidad Nacional Autónoma de México.
Cuernavaca, Mor.,
México*

1. Introduction

One major question in mammalian development is how the diverse cell types of the adult organism are generated from the initial population of undifferentiated cells in the preimplantation embryo. As part of this diversification process, the variety of neurons of the adult brain is generated from neural precursors specified during gastrulation and the posterior neurulation phase. The specification of neural precursors has a definitive influence on the fate of these cells and, consequently, in the neuron type derived after differentiation. Embryonic stem cells (ESCs), pluripotent cells derived from the inner cell mass of the blastocyst, can be used as a source of different neuron types *in vitro*, and the process of neuronal differentiation can thus be studied from the earliest stages starting from naïve non-neural undifferentiated cells. In particular, differentiation of ESCs into dopaminergic neurons has attracted a lot of attention for the relevance it may have in the design of cellular treatments for Parkinson's illness, a neurodegenerative disease characterized by the specific death of these neurons.

The midbrain dopaminergic (mDA) neurons constitute about 75% of all dopaminergic neurons in the adult brain (Wallen & Perlmann, 2003). They are located in the ventral region of the mesencephalon where they are organized laterally in the retrorubral field (RRF) and the substantia nigra pars compacta (SNc), and medially in the ventral tegmental area (VTA). The SNc neurons project to the dorsal striatum, forming the nigrostriatal pathway involved in the control of voluntary movements, and degeneration of this group of mDA neurons provokes the characteristic symptoms of Parkinson's disease (von Bohlen und Halbach, 2004). The neurons of the VTA project to the ventromedial striatum and the subcortical and cortical areas, forming the mesocortical limbic system, which is involved in emotional behaviors and mechanisms of motivation and reward. Misregulation of mesocortical limbic system has been involved in the development of drug addiction and depression (Kelley & Berridge, 2002), and contributes to certain symptoms of schizophrenia (Egan & Weinberger, 1997). Unlike SNc and VTA groups, RRF neurons have not been widely studied but it is known they project to the dorsal striatum and also connect SNc and VTA neurons (Arts et al., 1996).

There have been extensive efforts focused on the generation of mDA neurons from ESC, however, the limited success in this regard is largely due to our still only basic

understanding of the molecular mechanisms underlying mDA neuron development. Presently, it is known that the morphogens Sonic Hedgehog (Shh), Fibroblast growth factor 8 (Fgf8) and Wnt1 (Ye et al., 1998, Prakash et al., 2006) must act on early neural precursors at a particular dose and specific time window, as a failure to do so reduces or inhibits the correct differentiation process. It is known that the sequential and combinatory action of the morphogens activate at least two transcriptional factors, Lmx1a and Foxa2 that interact directly or indirectly to regulate the expression of other transcription factors promoting dopaminergic differentiation or inhibiting alternative neuronal fates (Nakatani et al., 2010, Lin et al., 2009, Ferri et al., 2007, Andersson et al., 2006). The complete potential of ESCs to differentiate into dopaminergic neurons is revealed once embryoid body (EB) cells derived from ESCs, are transplanted to explants of embryonic mesencephalon, where they efficiently and specifically respond to morphogens by differentiating and expressing the genes encoding the relevant transcription factors (Baizabal & Covarrubias, 2009).

Despite recent advances in understanding mDA development, the molecular interaction networks between the extrinsic and intrinsic factors involved are far from being fully understood. In the present chapter we will review how knowledge of mDA development has aided the refinement of differentiation protocols to generate mDA neurons from ESC and, how the ESC-based studies have contributed to understanding the mechanisms of differentiation into mDA neurons. We will discuss these data in the context of the differentiation potential of neural stem cells from different sources, the mechanism of stem cell specification, and the relevance that this has in defining the stem cell population and the differentiation conditions useful for the improvement of protocols for producing mDA neurons for the treatment of Parkinson Disease.

2. Development of midbrain dopaminergic neurons

Midbrain-DA neuronal differentiation initiates in the area rostral to the isthmus organizer and depends on the integration of anteroposterior and dorsoventral signals. These signals promote two related but distinct processes: specification of the general midbrain region, and specification of mDA precursor cells in particular (Fig.1). The distinction between these two processes is essential for the definition of the signaling cascade that leads to the generation of dopaminergic neurons.

The isthmus is formed at the mid-hindbrain border due to the complementary action of the *Otx2* and *Gbx2* genes in the anterior and posterior epiblast, respectively, at the end of gastrulation (E7.5) (Wurst & Bally-Cuif, 2001). Subsequently, *Pax2* is expressed at the interface of the *Otx2/Gbx2* domains, and *Wnt1* in the *Otx2*-positive territories. The expression of *Fgf8* starts at E8 at the caudal *Gbx2* expression domain and later restricts to the isthmus at around E9, forming a mirror image of the *Wnt1* expression domain. The activation of *Fgf8* expression is controlled by *Lmx1b* which, directly or indirectly, is also important for maintaining expression of other isthmus genes such as *Wnt1*, *En1/2*, *Pax2* and *Gbx2* (Guo et al., 2007). The genes encoding the transcription factors *En1/2* are expressed throughout the *Otx2/Gbx2* expression domain interface; there, they are required for the maintenance of *Fgf8* expression, though its early expression is independent of these two genes (Simon et al., 2004). Most of the morphogen activity of the isthmus is due to the products of *Fgf8* and *Wnt1* genes.

Crosstalk between the signals generated by *Fgf8* and *Wnt1* contributes to establish the identity and number of precursor cells committed to differentiate into mDA neurons (Lee et

al., 1997, Liu & Joyner, 2001, Matsunaga et al., 2002)(Fig. 1). In addition to their role in the formation of the midbrain, *Fgf8* and *Wnt1* seem to have a direct function in mDA neuron development. *Fgf8* regulates *Wnt1* expression in the isthmus (Chi et al., 2003) but an *Fgf* activity also controls *Wnt1* expression in the ventral midbrain as revealed in double and triple mutant knockout mice for the genes encoding two or three of the *Fgf* receptors (Saarimäki-Vire et al., 2007). Importantly, *Wnt1* is required for the ectopic induction of mDA neurons by *Fgf8* in the forebrain and hindbrain (Prakash et al., 2006).

At the same time as the isthmus is forming, *Shh* from the notochord induces the establishment of the floor plate (FP) along the neural tube (Yamada et al., 1991) (Fig.1). *Foxa2* expression in FP cells, via *Shh* activity-dependent *Gli2* transcription factor binding sites in the *Foxa2* promoter (Sasaki et al., 1997), is required for the formation of the FP (Matise et al., 1998). *Foxa2*, in turn, directly induces *Shh* expression in ventral domains of mesencephalon where is essential for the specification of ventral neural precursors (Jeong & Epstein, 2003). Fate-mapping experiments clearly establish that cells expressing *Shh* in the FP become mDA neurons (Ono et al., 2007). This is a unique characteristic of mDA neurons, as at other regions along the ventral neural tube, FP cells are non-neurogenic (Joksimovic et al., 2009; Ono, et al., 2007). As expected, lack of *Foxa2* and the related transcription factor *Foxa1* reduces the number of mDA neurons produced in the midbrain (Lin et al., 2009).

Lmx1a and *Lmx1b* are among the first markers that identify the mDA precursors and are key regulators of their differentiation. As with *Fgf8* and *Wnt1*, *Lmx1b* has two separate roles; one is related to the formation and maintenance of the isthmus, as described above, and, consequently is essential for midbrain development; whereas the other seems to be directly involved in mDA neuron generation as *Lmx1b* expression is detected before emergence of mDA neurons (E7.5) and is maintained in the SNc and VTA until adulthood (Smidt et al., 2000). On the other hand, *Lmx1a*, whose gene is expressed in ventral midline of mesencephalon around E9, appears essential for mDA differentiation in the chick and its absence causes a reduction in dopaminergic neurons in the mouse (Ono et al., 2007, Andersson et al., 2006). *Otx2* is necessary for the expression of *Lmx1a* in FP cells but, apparently, not for *Lmx1b* (Omodei et al., 2008, Ono et al., 2007). In addition, recent data suggest that *Foxa2* is also required to induce and/or to maintain *Lmx1a* and *Lmx1b* expression (Lin et al., 2009). Therefore, *Foxa2* and *Otx2* may cooperatively function to define *Lmx1a*, and possibly also *Lmx1b*, expression in ventral midbrain precursors. Until now, it is not clear if *Lmx1a* and *Lmx1b* work in independent pathways or if they have redundant activities (see below).

Downstream of *Shh* signaling, *Foxa1/2* inhibit the expression of the gene encoding *Nkx2.2* (Lin et al., 2009), a transcription factor present adjacent to the *Lmx1a* domain at early stages, and later between the dorsal and ventral *Nkx6.1* domains in a narrow band that eventually give rise to GABAergic neurons (Nakatani et al., 2007)(Fig.1). On the other hand, *Nkx6.1* is present in the mDA domain at the beginning of *Lmx1a* expression, but later its gene is repressed by *Msx1*; the *Shh* secreted by the FP activates *Msx1* expression in the ventral midbrain (Andersson et al., 2006). Thus, *Nkx6.1* expression only remains in the regions adjacent to the *Lmx1a* domain, where motoneurons are generated (Fedtsova & Turner, 2001).

The generation of mDA neurons must be coordinated with the conversion of FP cells into neuronal progenitors. This transition might be due to the action of the canonical *Wnt* signaling, activated by *Otx2* in the midbrain (Ono et al., 2007, Omodei et al., 2008)(Fig. 1). Around E10.5, *Wnt1* suppresses *Shh* expression levels, possibly via an *Msx1*-mediated

mechanism (Joksimovic et al., 2009), and consequently, induces the expression of *Ngn2*, encoding Neurogenin 2, a panneural basic helix-loop-helix protein (Ono et al., 2007). *Foxa1/2* apparently also contribute to the activation of *Ngn2* expression (Ferri et al., 2007), which reinforces this mDA differentiation regulatory pathway. The induction of *Ngn2* expression together with that of *Mash1* mark the initiation of neurogenesis itself (Kele et al., 2006)(Fig. 1). Neurogenesis is immediately followed by the expression of *Nurr1* and *Pitx3*, genes encoding transcription factors that are involved in defining the dopaminergic phenotypic characteristics such as the presence of tyrosine hydroxylase (the rate-limiting enzyme in dopamine synthesis; TH), vesicular monoamine transporter 2 (Vmat2) and dopamine transporter (DAT) (Zetterstrom et al., 1997, Smits et al., 2003, Saucedo-Cardenas et al., 1998, Kim et al., 2003b, Ferri et al., 2007)(Fig. 1). Also, *Nurr1* and *Pitx3* appear to play a role in the maintenance of the mDA neurons (Smidt et al., 2004, Nunes et al., 2003). *Foxa1/2* function cooperatively with *Lmx1a* to regulate *Nurr1* expression in immature neurons, and also directly activate *Th* expression in mature neurons (Fig. 1). Therefore, it is possible that *Nurr1* and *Foxa2* cooperate to regulate *Th* expression during mDA differentiation. *En1* and *En2*, in addition to their participation in the establishment of the mid-hindbrain border at early stages, also play an important role in the survival of mDA neurons (Alberi et al., 2004, Simon et al., 2001, Simon et al., 2004).

Fig. 1. Molecular network controlling mDA differentiation. (A) Frontal view representation of the embryonic mesencephalon in which the distinct regulatory events during mDA neuron development are shown. (B) Interactions between the distinct determinants of the key events that lead specific mDA differentiation during development (represented by the same colors as in A). The dashed arrows represent indirect or unconfirmed regulations. IsO, Isthmus organizer; FP, floor plate.

3. Neuralization and specification of ESC

The capacity of ESC to generate all cell types present in the embryo has made them a potential source of different neuron types *in vitro*, and so the process of specific neural differentiation can be studied since its very first stage and manipulated in an easier and faster manner than in embryos.

Ectodermal differentiation of ESC through EB formation is commonly used to produce neural precursor cells (NPCs) *in vitro*. The formation of EBs reflects the early developmental stages that lead to the formation of the epiblast and the primitive endoderm, immediately previous to gastrulation. EB cells can be neuralized with retinoic acid and produce about 40% of neurons of several types (Fraichard et al., 1995, Bain et al., 1995). Alternatively, EB cells can be cultured in a neural-defined medium, and the NPC population be expanded with Fgf2; under these conditions, more than 70% of the total cells are NPC which can be differentiated into neurons by removing Fgf2 (Okabe et al., 1996). Retinoic acid not only induces neuralization, but also instructs the cells to posterior fates (Okada et al., 2004), which limits the neuron types that can be derived from EBs; this potential problem can be overcome by treating the cells with low doses of RA. In fact, endogenous RA appears to be required for the neuralization in the absence of a direct RA addition to the medium (Engberg et al., 2010). Bmp signaling represses neural fates *in vivo* (Hemmati-Brivanlou & Melton, 1997, Finley et al., 1999); in concordance, Noggin and Chordin, two Bmp inhibitors, induce neural differentiation of EB cells (Gratsch & O'Shea, 2002). Another condition that promotes neural differentiation of EB cells is to block the Wnt/ β -catenin pathway by adding its inhibitor Dkk-1 (Verani et al., 2007) or in the absence of Wnt and Nodal in cultures of EB cells in serum-free conditions (Watanabe et al., 2005).

Neuronal differentiation of ESC can also be induced without forming EBs. One protocol involves culturing the cells at low densities in a chemically defined, serum-free medium with LIF and Fgf2 (Trobepe et al., 2001). The cells of the spherical colonies formed in these conditions express the neural marker Nestin, and can differentiate further into neurons and glia; of note is that even though all cells in the colonies are NPC, very few of the cultured ESC generate colonies (0.2%). It has been proposed that the colonies represent primitive neuroectodermal cells, as similar colonies can be obtained from the embryo (Hitoshi et al., 2004). A similar protocol to generate neural precursors without the formation of EBs is to grow the ESCs as a monolayer at low density in serum-free conditions with N2B27 medium (Ying et al., 2003). After 4 days, 60% of the cells express *Sox1*, one of the first markers of neuroectodermal cells and these *Sox1*-positive cells, can later differentiate into mature neurons. This latter protocol could represent a simpler and direct system to assess neural differentiation and specification as exogenous growth factors and the formation of EB are not required.

In order to generate a particular type of neurons, it is necessary not only to neuralize the cells but also to expose them to other factors that promote specific neuronal differentiation. For instance, treating EBs with RA (with neuralizing and posteriorizing activities) and Shh (with ventralizing activity) oblige the cells to differentiate into motor neurons of the spinal cord (Wichterle et al., 2002). On the other hand, neural cells generated after inhibition of Wnt and Nodal signaling acquire a telencephalic identity (expression of *Foxg1*), and these neural cells can later be differentiated into subpopulations of telencephalic neurons depending on whether Wnt1 (forming pallial telencephalic neurons *Foxg1*⁺*Pax6*⁺) or Shh (basal telencephalic neurons *Foxg1*⁺*Nkx2.1*⁺) is added to the culture medium (Watanabe et al., 2005).

The fact that, in different culture conditions that promote ESC neuronal differentiation, NPCs emerge prior to neuron formation (as determined by the expression of *Sox1*, *Sox2* and *Nestin*), and that at least some of these NPCs respond to morphogenetic cues, suggest that ESC neural differentiation *in vitro* follows a comparable program to that occurring *in vivo*. Therefore, ESCs are useful as a model to study early neural differentiation.

4. Specification and differentiation of ESCs into mDA neurons

4.1 Response of ESC-derived cells to extrinsic determinants

Shh and Fgf8 were the first morphogens identified that are sufficient to induce the generation of ectopic mDA neurons in explant cultures (Ye et al., 1998). Expectedly, these morphogens were among the first used to induce mDA differentiation of ESC *in vitro* (Fig. 2). EB cells cultured in the absence of growth factors, and the enriched NPC population subsequently expanded in the presence of Fgf8 and Shh, produce between 10-20% of mDA neurons after allowing neuronal differentiation, in comparison with the 1-2% of mDA neurons produced in their absence (expressed as a percentage of the total cells alive at the end of the culture) (Rodriguez-Gomez et al., 2007, Lee et al., 2000, Hedlund et al., 2008, Kim et al., 2002). Although the addition of Shh and Fgf8 after EB formation increases the number of mDA neurons produced, the proportion of mDA neurons generated is still generally low. A similar proportion of mDA neurons are obtained when ESC are grown on a monolayer of PA6 cells (stromal cells derived from skull marrow) (Kawasaki et al., 2000), or when ESC neuralized directly in monolayer culture are treated with Fgf8 and Shh (Ying et al., 2003) (Fig. 2). It is apparent from these data that the population of cells capable of responding to specific environmental cues in culture is limited and/or the culture conditions lack the relevant signals present in the embryonic mesencephalic niche necessary for the efficient differentiation. Alternatively, the time window of action of each growth factor needs to be reproduced as *in vivo* in order to get efficient specific differentiation.

Although ESC are pluripotent and have the full potential to derive into any neuron type, common neuralization protocols might limit the cells ability to differentiate into dopaminergic neurons. This is supported by the fact that most ESC-derived NPCs are unable to respond to cues present in the niche of endogenous differentiation dopaminergic neuron differentiation (Baizabal & Covarrubias, 2009). Fgf8 and Shh do not alter the differentiation potential of the neuralized ESC and only induce the specific dopaminergic differentiation of a small proportion of cells. However, when non-neuralized EB cells are exposed to the embryonic mesencephalic environment, efficient and specific differentiation results (Baizabal & Covarrubias, 2009) (Fig. 2). Therefore, the time window that allows proper dopaminergic specification appears to be around the time at which the cells neuralize.

Since there is no certainty that the adult brain has the proper environment for specific differentiation, transplantation of EB cells at this stage is not the proper way to evaluate their differentiation potential. Nonetheless, differentiation into dopaminergic neurons has been achieved when EB cells are transplanted to the striatum of the adult brain, the target region of dopaminergic neurons of substantia nigra. As expected only few dopaminergic neurons are found several weeks after implantation, and those neurons do not apparently result from induction processes occurring in the adult brain. In fact, the proportion of dopaminergic and serotonergic neurons is similar to that obtained *in vitro* or when the cells are transplanted to the kidney capsule (Bjorklund et al., 2002, Deacon et al., 1998).

Transplantation to the embryonic brain could provide a more adequate milieu for specific differentiation of ESC or their derivatives. ESC-derived neural precursors transplanted to the embryonic and neonatal brain efficiently differentiate into neurons and glial cells (Brustle et al., 1997, Wernig et al., 2004, Zhang et al., 2001). However, some ESC-derived neurons that expressed *Th* and *En1* can be found outside the midbrain (Wernig et al., 2004) suggesting that the cells are already committed before transplantation. In the previous type of experiments, cells are transplanted to embryos at stages long after specification of endogenous dopaminergic precursors has completed (E13), and therefore, the environment may not be the best suited to the induction of the dopaminergic differentiation. In contrast, robust specification and differentiation results when EB cells are transplanted to explants of E10.5 mesencephalon, just 1-2 days after specification of the endogenous mDA NPCs. In this condition, nearly 70% of the cells acquire neuronal markers and, of the total transplanted cells, 65% express *Lmx1a* and about 40% *Th* (Baizabal & Covarrubias, 2009). Therefore, at least up to E10.5, all the signals required to instruct EB cells into different mesencephalic phenotypes are present.

Fig. 2. Schematic representation of diverse strategies used to generate mDA neurons from ESCs.

4.2 Intrinsic determinants

As more is discovered about the transcription factors that control dopaminergic differentiation, the possibilities to induce specific differentiation through the overexpression of one or more transcription factors have increased. It might be predicted that the ectopic expression of a master gene(s) will be sufficient to induce specific neuronal differentiation programmes. However, the success in the searching for such “master genes” in general has been rather low, perhaps due to the requirement for a specific combination of transcription factors to be expressed in a competent recipient stem cell. The ‘strength’ of a master gene can be defined as a function of its ability to promote specific differentiation of precursor cells from the earliest stages possible. In this sense, the ‘strongest’ master gene will be that which individually promotes the specific differentiation of undifferentiated ESCs. It is apparent that none of the transcription factors of the dopaminergic network have the ability to induce specific differentiation following expression in undifferentiated ESCs.

Overproduction of transcription factors acting at late stages of dopaminergic differentiation (dopaminergic determinants) have been tested in different cell culture systems. Overexpression of Nurr1 in embryonic NPC from distinct regions of the brain cultured as a monolayer or after forming neurospheres, induces TH expression (Kim et al., 2003a, Kim et al., 2007); however, other characteristics of dopaminergic neurons have not been reported. When overexpression of Nurr1 is induced in NPC generated from ESCs, TH and other dopaminergic neuronal markers, such as DAT, are detected, however, Nurr1 has no influence over transcription factors important for mDA specification (Martinat et al., 2006, Sonntag et al., 2004). Overexpression of Pitx3 in undifferentiated ESC or in ESC-derived neural precursors is not sufficient to induce the complete mDA phenotype, but promotes expression of a subset of markers, particularly Aldh2 (Martinat et al., 2006, Chung et al., 2005). Even Nurr1 and Pitx3 together are not sufficient to induce ESC mDA differentiation and again, they only appear to influence terminal dopaminergic maturation (Martinat et al., 2006). The previous observations are in agreement with a direct role of these transcription factors in the expression of the *Th* gene and other genes that contribute to the dopaminergic phenotype. Nonetheless, an effect of Nurr1 on dopaminergic differentiation appears to occur when is constitutively overexpressed during the differentiation of ESC in the presence of Fgf8 and Shh as it enhances approximately four-fold the quantity of dopaminergic neurons generated (Kim et al., 2002).

Several factors relevant for the neuralization and specification of mDA NPCs have been overexpressed. Overexpression of Msx1 alone in ESC does not induce the generation of mDA neurons, though it is sufficient to induce *Ngn2* expression as it appears to occur *in vivo* (Andersson et al., 2006). In contrast, the overexpression of Lmx1a under the control of the Nestin promoter in ESC treated with Fgf8 and Shh causes an extensive increase in cells expressing *Msx1* and a reduction in those expressing *Nkx6.1* (Fig. 2). Also, there is a robust generation of immature neurons that express *Th*, many of which also express additional genes encoding mDA neuronal markers, including Lmx1a, Lmx1b, En1/2, Nurr1, Pitx3, Foxa2, DAT and Vmat2 (Friling et al., 2009, Andersson et al., 2006). This highly specific differentiation is observed under two distinct *in vitro* protocols suggesting that the effects are due to the action of Lmx1a and not to particular culture conditions. In agreement with a key role for Lmx1a also in human mDA neuron development, overexpression of Lmx1a under the control of the nestin promoter in human ESC generated abundant TH⁺ cells (Friling et al., 2009). It is worth mentioning that Lmx1a and other transcription factors within the dopaminergic specification network are not able to promote specific

differentiation when overexpressed in neurospheres cells, suggesting that these NPCs are not competent to respond to these specification factors (Roybon et al., 2008).

In the previous protocols, the use of the nestin promoter to drive *Lmx1a* expression appears key for the efficient mDA differentiation of ESC-derived neural precursors. The exogenous *Lmx1a* initiates its expression at the time Nestin⁺ neural precursors first emerge in culture (Andersson et al., 2006, Friling et al., 2009), which may provide the right time window for the action of *Lmx1a* in which ESC-derived neural precursors are competent to specifically respond and generate mDA neurons. These data reinforces the idea mentioned above proposing that specification and neuralization are interdependent process that must occur simultaneously or within a narrow window during the differentiation process.

Recently, studies with ESC have revealed novel interactions among the different factors participating in mDA differentiation. Based on the transcription factor network determined during mDA development, different combinations of *Foxa2*, *Lmx1a* and *Otx2* have been introduced into ESC-derived neural precursors and the effect on mDA differentiation determined. When genes encoding the three factors are ectopically expressed in the absence of *Shh* in the culture medium, robust synergistic induction of mDA markers is observed with the majority of TH⁺ neurons also coexpressing *Lmx1b* and *Nurr1* (Chung et al., 2009). This highly efficient generation of mDA neurons is not observed when *Foxa2* or *Otx2* are introduced alone. Therefore, it is apparent that *Lmx1a* is the key specification determinant of mDA differentiation in ESCs that, in concert with *Otx2*, could give a midbrain identity to neural precursor cells. *Foxa2* might be required to activate the *Shh* pathway relevant for efficient mDA differentiation.

Studies with ESCs have identified a *Wnt1*-*Lmx1a/b* loop relevant for the specification of mDA neurons. In ESC *Wnt1* induces the activation of *Lmx1a* independent of both *Shh* and *Otx2*, whereas, *Lmx1a* and *Lmx1b* upregulate *Wnt1* by binding directly to its promoter (Chung et al., 2009). This autoregulatory loop, in turn, directly regulates *Otx2* expression, through the canonical *Wnt* signaling pathway, and also *Nurr1* and *Pitx3* expression through *Lmx1a* and *Lmx1b*. The *Wnt1*-*Lmx1a/b* loop also functions in the embryonic midbrain (Chung et al., 2009), demonstrating that this is not a culture artifact and that ESCs is a suitable system to study mDA differentiation. In this system, *Lmx1a* and *Lmx1b* appear to regulate each other, and the absence of one compensates the function of the other in the regulation of *Wnt1*, *Nurr1*, *Pitx3* and *Th* expression (Chung et al., 2009).

5. Conclusions

Taking together the data from *in vivo* and *in vitro* studies, at least two major pathways appear to regulate mDA specification, which may be referred to as “*Shh-Foxa2*” and “*Wnt1-Lmx1a/b*” (Fig. 1). These pathways, to some extent, are independent, as *Wnt1* or *Lmx1a* do not affect *Shh* or *Foxa2* expression; although maintainance of *Lmx1a* expression is dependent on *Foxa2* activity. At a later stage, these two pathways converge on *Nurr1* and dopaminergic differentiation itself initiates.

Most stem cell-based differentiation protocols are focused on the addition of growth factors to the culture medium, in part because genetic manipulations are generally not desirable in the generation of cells for therapeutic purposes (Fig. 2). However, finding the right combination of growth factors and the time of action, as well as the competent stem cell population responsive to those factors is a difficult task. On the other hand, as we learn more about the transcriptional network that control specific fates, the use of “master genes”

becomes more attractive. Therefore, engineering ESC lines that overproduce transcription factor(s) required for mDA development, in addition to the relevant basic information provided for the understanding of the molecular mechanisms involved in dopaminergic differentiation, it presents a promising alternative to obtain authentic mDA neurons for treating Parkinson's disease (Fig. 3).

It does not appear to be far the time at which there are one or several protocols to produce efficiently mDA neurons *in vitro*. However, the development of a cell therapy adequate for the treatment of patients suffering Parkinson's disease will require more than a protocol to produce mDA neurons, considering that it is unlikely that the adult brain possesses the optimal environment for mDA NPC differentiation and neuron maturation. For instance, it will be relevant to determine the differentiation stage at which implantation and survival of the transplanted cells is more efficient, as well as to identify additional factors needed to reconstitute the nigrostriatal pathway.

Fig. 3. ESC-based therapies for Parkinson's disease. ESCs are a potential source of mDA neurons useful in cell therapies for Parkinson's disease. The efficiency to generate mDA neurons from ESCs is increasing as we learn more about the factors controlling mDA neuronal differentiation from studies with both embryos and ESCs.

6. Acknowledgements

We thank to present and past members of the Laboratory on Tissue Degeneration and Regeneration for the fruitful discussions that contributed to develop some ideas presented in this review. We are particularly grateful with Christopher Wood for critical reading of the

manuscript. This work was supported by Project Program Grant IMPULSA-UNAM 03 and Consejo Nacional de Ciencia y Tecnología (50956-Q).

7. References

- Alberi, L., Sgado, P. & Simon, H. H. (2004). Engrailed genes are cell-autonomously required to prevent apoptosis in mesencephalic dopaminergic neurons. *Development*. 131, (Jul 2004) 3229-36, 0950-1991
- Andersson, E., Tryggvason, U., Deng, Q., Friling, S., Alekseenko, Z., Robert, B., Perlmann, T. & Ericson, J. (2006). Identification of intrinsic determinants of midbrain dopamine neurons. *Cell*. 124, (Jan 2006) 393-405, 0092-8674
- Arts, M. P., Groenewegen, H. J., Veening, J. G. & Cools, A. R. (1996). Efferent projections of the retrorubral nucleus to the substantia nigra and ventral tegmental area in cats as shown by anterograde tracing. *Brain Res Bull*. 40, 1996) 219-28, 0361-9230
- Bain, G., Kitchens, D., Yao, M., Huettner, J. E. & Gottlieb, D. I. (1995). Embryonic stem cells express neuronal properties in vitro. *Dev Biol*. 168, (Apr 1995) 342-57, 0012-1606
- Baizabal, J. M. & Covarrubias, L. (2009). The embryonic midbrain directs neuronal specification of embryonic stem cells at early stages of differentiation. *Dev Biol*. 325, (Jan 1 2009) 49-59, 0012-1606
- Bjorklund, L. M., Sanchez-Pernaute, R., Chung, S., Andersson, T., Chen, I. Y., McNaught, K. S., Brownell, A. L., Jenkins, B. G., Wahlestedt, C., Kim, K. S. & Isacson, O. (2002). Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A*. 99, (Feb 2002) 2344-9, 0027-8424
- Brustle, O., Spiro, A. C., Karram, K., Choudhary, K., Okabe, S. & McKay, R. D. (1997). In vitro-generated neural precursors participate in mammalian brain development. *Proc Natl Acad Sci U S A*. 94, (Dec 23 1997) 14809-14, 0027-8424
- Chi, C. L., Martinez, S., Wurst, W. & Martin, G. R. (2003). The isthmic organizer signal FGF8 is required for cell survival in the prospective midbrain and cerebellum. *Development*. 130, (Jun 2003) 2633-44, 0950-1991
- Chung, S., Hedlund, E., Hwang, M., Kim, D. W., Shin, B. S., Hwang, D. Y., Jung Kang, U., Isacson, O. & Kim, K. S. (2005). The homeodomain transcription factor Pitx3 facilitates differentiation of mouse embryonic stem cells into AHD2-expressing dopaminergic neurons. *Mol Cell Neurosci*. 28, (Feb 2005) 241-52, 1044-7431
- Chung, S., Leung, A., Han, B. S., Chang, M. Y., Moon, J. I., Kim, C. H., Hong, S., Pruszak, J., Isacson, O. & Kim, K. S. (2009). Wnt1-lmx1a forms a novel autoregulatory loop and controls midbrain dopaminergic differentiation synergistically with the SHH-FoxA2 pathway. *Cell Stem Cell*. 5, (Dec 2009) 646-58, 1875-9777
- Deacon, T., Dinsmore, J., Costantini, L. C., Ratliff, J. & Isacson, O. (1998). Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp Neurol*. 149, (Jan 1998) 28-41, 0014-4886
- Egan, M. F. & Weinberger, D. R. (1997). Neurobiology of schizophrenia. *Curr Opin Neurobiol*. 7, (Oct 1997) 701-7, 0959-4388
- Engberg, N., Kahn, M., Petersen, D. R., Hansson, M. & Serup, P. (2010). Retinoic acid synthesis promotes development of neural progenitors from mouse embryonic stem cells by suppressing endogenous, Wnt-dependent nodal signaling. *Stem Cells*. 28, (Sep 2010) 1498-509, 1066-5099

- Fedtsova, N. & Turner, E. E. (2001). Signals from the ventral midline and isthmus regulate the development of Brn3.0-expressing neurons in the midbrain. *Mech Dev.* 105, (Jul 2001) 129-44, 0925-4773
- Ferri, A. L., Lin, W., Mavromatakis, Y. E., Wang, J. C., Sasaki, H., Whitsett, J. A. & Ang, S. L. (2007). Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. *Development.* 134, (Aug 2007) 2761-9, 0950-1991
- Finley, M. F., Devata, S. & Huettner, J. E. (1999). BMP-4 inhibits neural differentiation of murine embryonic stem cells. *J Neurobiol.* 40, (Sep 1999) 271-87, 0022-3034
- Fraichard, A., Chassande, O., Bilbaut, G., Dehay, C., Savatier, P. & Samarut, J. (1995). In vitro differentiation of embryonic stem cells into glial cells and functional neurons. *J Cell Sci.* 108 (Pt 10), (Oct 1995) 3181-8, 0021-9533
- Friling, S., Andersson, E., Thompson, L. H., Jonsson, M. E., Hebsgaard, J. B., Nanou, E., Alekseenko, Z., Marklund, U., Kjellander, S., Volakakis, N., Hovatta, O., El Manira, A., Bjorklund, A., Perlmann, T. & Ericson, J. (2009). Efficient production of mesencephalic dopamine neurons by Lmx1a expression in embryonic stem cells. *Proc Natl Acad Sci U S A.* 106, (May 2009) 7613-8, 0027-8424
- Gratsch, T. E. & O'Shea, K. S. (2002). Noggin and chordin have distinct activities in promoting lineage commitment of mouse embryonic stem (ES) cells. *Dev Biol.* 245, (May 2002) 83-94, 0012-1606
- Guo, C., Qiu, H. Y., Huang, Y., Chen, H., Yang, R. Q., Chen, S. D., Johnson, R. L., Chen, Z. F. & Ding, Y. Q. (2007). Lmx1b is essential for Fgf8 and Wnt1 expression in the isthmus organizer during tectum and cerebellum development in mice. *Development.* 134, (Jan 2007) 317-25, 0950-1991
- Hedlund, E., Pruszak, J., Lardaro, T., Ludwig, W., Vinuela, A., Kim, K. S. & Isacson, O. (2008). Embryonic stem cell-derived Pitx3-enhanced green fluorescent protein midbrain dopamine neurons survive enrichment by fluorescence-activated cell sorting and function in an animal model of Parkinson's disease. *Stem Cells.* 26, (Jun 2008) 1526-36, 1066-5099
- Hemmati-Brivanlou, A. & Melton, D. (1997). Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell.* 88, (Jan 1997) 13-7, 0092-8674
- Hitoshi, S., Seaberg, R. M., Kosciuk, C., Alexson, T., Kusunoki, S., Kanazawa, I., Tsuji, S. & van der Kooy, D. (2004). Primitive neural stem cells from the mammalian epiblast differentiate to definitive neural stem cells under the control of Notch signaling. *Genes Dev.* 18, (Aug 2004) 1806-11, 0890-9369
- Jeong, Y. & Epstein, D. J. (2003). Distinct regulators of Shh transcription in the floor plate and notochord indicate separate origins for these tissues in the mouse node. *Development.* 130, (Aug 2003) 3891-902, 0950-1991
- Joksimovic, M., Yun, B. A., Kittappa, R., Anderregg, A. M., Chang, W. W., Taketo, M. M., McKay, R. D. & Awatramani, R. B. (2009). Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. *Nat Neurosci.* 12, (Feb 2009) 125-31, 1097-6256
- Kawasaki, H., Mizuseki, K., Nishikawa, S., Kaneko, S., Kuwana, Y., Nakanishi, S., Nishikawa, S. I. & Sasai, Y. (2000). Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron.* 28, (Oct 2000) 31-40, 0896-6273

- Kele, J., Simplicio, N., Ferri, A. L., Mira, H., Guillemot, F., Arenas, E. & Ang, S. L. (2006). Neurogenin 2 is required for the development of ventral midbrain dopaminergic neurons. *Development*. 133, (Feb 2006) 495-505, 0950-1991
- Kelley, A. E. & Berridge, K. C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci*. 22, (May 1 2002) 3306-11, 0270-6474
- Kim, H. J., Sugimori, M., Nakafuku, M. & Svendsen, C. N. (2007). Control of neurogenesis and tyrosine hydroxylase expression in neural progenitor cells through bHLH proteins and Nurr1. *Exp Neurol*. 203, (Feb 2007) 394-405, 0014-4886
- Kim, J. H., Auerbach, J. M., Rodriguez-Gomez, J. A., Velasco, I., Gavin, D., Lumelsky, N., Lee, S. H., Nguyen, J., Sanchez-Pernaute, R., Bankiewicz, K. & McKay, R. (2002). Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature*. 418, (Jul 2002) 50-6, 0028-0836
- Kim, J. Y., Koh, H. C., Lee, J. Y., Chang, M. Y., Kim, Y. C., Chung, H. Y., Son, H., Lee, Y. S., Studer, L., McKay, R. & Lee, S. H. (2003a). Dopaminergic neuronal differentiation from rat embryonic neural precursors by Nurr1 overexpression. *J Neurochem*. 85, (Jun 2003a) 1443-54, 0022-3042
- Kim, K. S., Kim, C. H., Hwang, D. Y., Seo, H., Chung, S., Hong, S. J., Lim, J. K., Anderson, T. & Isacson, O. (2003b). Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. *J Neurochem*. 85, (May 2003b) 622-34, 0022-3042
- Lee, J., Platt, K. A., Censullo, P. & Ruiz i Altaba, A. (1997). Gli1 is a target of Sonic hedgehog that induces ventral neural tube development. *Development*. 124, (Jul 1997) 2537-52, 0950-1991
- Lee, S. H., Lumelsky, N., Studer, L., Auerbach, J. M. & McKay, R. D. (2000). Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol*. 18, (Jun 2000) 675-9, 1087-0156
- Lin, W., Metzakopian, E., Mavromatakis, Y. E., Gao, N., Balaskas, N., Sasaki, H., Briscoe, J., Whitsett, J. A., Goulding, M., Kaestner, K. H. & Ang, S. L. (2009). Foxa1 and Foxa2 function both upstream of and cooperatively with Lmx1a and Lmx1b in a feedforward loop promoting mesodiencephalic dopaminergic neuron development. *Dev Biol*. 333, (Sep 2009) 386-96, 0012-1606
- Liu, A. & Joyner, A. L. (2001). EN and GBX2 play essential roles downstream of FGF8 in patterning the mouse mid/hindbrain region. *Development*. 128, (Jan 2001) 181-91, 0950-1991
- Martinat, C., Bacci, J. J., Leete, T., Kim, J., Vanti, W. B., Newman, A. H., Cha, J. H., Gether, U., Wang, H. & Abeliovich, A. (2006). Cooperative transcription activation by Nurr1 and Pitx3 induces embryonic stem cell maturation to the midbrain dopamine neuron phenotype. *Proc Natl Acad Sci U S A*. 103, (Feb 2006) 2874-9, 0027-8424
- Matise, M. P., Epstein, D. J., Park, H. L., Platt, K. A. & Joyner, A. L. (1998). Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. *Development*. 125, (Aug 1998) 2759-70, 0950-1991
- Matsunaga, E., Katahira, T. & Nakamura, H. (2002). Role of Lmx1b and Wnt1 in mesencephalon and metencephalon development. *Development*. 129, (Nov 2002) 5269-77, 0950-1991
- Nakatani, T., Kumai, M., Mizuhara, E., Minaki, Y. & Ono, Y. (2010). Lmx1a and Lmx1b cooperate with Foxa2 to coordinate the specification of dopaminergic neurons and

- control of floor plate cell differentiation in the developing mesencephalon. *Dev Biol.* 339, (Mar 1 2010) 101-13, 0012-1606
- Nakatani, T., Minaki, Y., Kumai, M. & Ono, Y. (2007). Helt determines GABAergic over glutamatergic neuronal fate by repressing Ngn genes in the developing mesencephalon. *Development.* 134, (Aug 2007) 2783-93, 0950-1991
- Nunes, I., Tovmasian, L. T., Silva, R. M., Burke, R. E. & Goff, S. P. (2003). Pitx3 is required for development of substantia nigra dopaminergic neurons. *Proc Natl Acad Sci U S A.* 100, (Apr 2003) 4245-50, 0027-8424
- Okabe, S., Forsberg-Nilsson, K., Spiro, A. C., Segal, M. & McKay, R. D. (1996). Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. *Mech Dev.* 59, (Sep 1996) 89-102, 0925-4773
- Okada, Y., Shimazaki, T., Sobue, G. & Okano, H. (2004). Retinoic-acid-concentration-dependent acquisition of neural cell identity during in vitro differentiation of mouse embryonic stem cells. *Dev Biol.* 275, (Nov 2004) 124-42, 0012-1606
- Omodei, D., Acampora, D., Mancuso, P., Prakash, N., Di Giovannantonio, L. G., Wurst, W. & Simeone, A. (2008). Anterior-posterior graded response to Otx2 controls proliferation and differentiation of dopaminergic progenitors in the ventral mesencephalon. *Development.* 135, (Oct 2008) 3459-70, 0950-1991
- Ono, Y., Nakatani, T., Sakamoto, Y., Mizuhara, E., Minaki, Y., Kumai, M., Hamaguchi, A., Nishimura, M., Inoue, Y., Hayashi, H., Takahashi, J. & Imai, T. (2007). Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development.* 134, (Sep 2007) 3213-25, 0950-1991
- Prakash, N., Brodski, C., Naserke, T., Puelles, E., Gogoi, R., Hall, A., Panhuysen, M., Echevarria, D., Sussel, L., Weisenhorn, D. M., Martinez, S., Arenas, E., Simeone, A. & Wurst, W. (2006). A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors in vivo. *Development.* 133, (Jan 2006) 89-98, 0950-1991
- Rodriguez-Gomez, J. A., Lu, J. Q., Velasco, I., Rivera, S., Zoghbi, S. S., Liow, J. S., Musachio, J. L., Chin, F. T., Toyama, H., Seidel, J., Green, M. V., Thanos, P. K., Ichise, M., Pike, V. W., Innis, R. B. & McKay, R. D. (2007). Persistent dopamine functions of neurons derived from embryonic stem cells in a rodent model of Parkinson disease. *Stem Cells.* 25, (Apr 2007) 918-28, 1066-5099
- Roybon, L., Hjalt, T., Christophersen, N. S., Li, J. Y. & Brundin, P. (2008). Effects on differentiation of embryonic ventral midbrain progenitors by Lmx1a, Msx1, Ngn2, and Pitx3. *J Neurosci.* 28, (Apr 2008) 3644-56, 0270-6474
- Saarimäki-Vire, J., Peltopuro, P., Lahti, L., Naserke, T., Blak, A. A., Vogt Weisenhorn, D. M., Yu, K., Ornitz, D. M., Wurst, W. & Partanen, J. (2007). Fibroblast growth factor receptors cooperate to regulate neural progenitor properties in the developing midbrain and hindbrain. *J Neurosci.* 27, (Aug 2007) 8581-92, 0270-6474
- Sasaki, H., Hui, C., Nakafuku, M. & Kondoh, H. (1997). A binding site for Gli proteins is essential for HNF-3 β floor plate enhancer activity in transgenics and can respond to Shh in vitro. *Development.* 124, (Apr 1997) 1313-22, 0950-1991
- Saucedo-Cardenas, O., Quintana-Hau, J. D., Le, W. D., Smidt, M. P., Cox, J. J., De Mayo, F., Burbach, J. P. & Conneely, O. M. (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late

- dopaminergic precursor neurons. *Proc Natl Acad Sci U S A*. 95, (Mar 1998) 4013-8, 0027-8424
- Simon, H. H., Saueressig, H., Wurst, W., Goulding, M. D. & O'Leary, D. D. (2001). Fate of midbrain dopaminergic neurons controlled by the engrailed genes. *J Neurosci*. 21, (May 2001) 3126-34, 0270-6474
- Simon, H. H., Thuret, S. & Alberi, L. (2004). Midbrain dopaminergic neurons: control of their cell fate by the engrailed transcription factors. *Cell Tissue Res*. 318, (Oct 2004) 53-61, 0302-766X
- Smidt, M. P., Asbreuk, C. H., Cox, J. J., Chen, H., Johnson, R. L. & Burbach, J. P. (2000). A second independent pathway for development of mesencephalic dopaminergic neurons requires Lmx1b. *Nat Neurosci*. 3, (Apr 2000) 337-41, 1097-6256
- Smidt, M. P., Smits, S. M., Bouwmeester, H., Hamers, F. P., van der Linden, A. J., Hellemons, A. J., Graw, J. & Burbach, J. P. (2004). Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development*. 131, (Mar 2004) 1145-55, 0950-1991
- Smits, S. M., Ponnio, T., Conneely, O. M., Burbach, J. P. & Smidt, M. P. (2003). Involvement of Nurr1 in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. *Eur J Neurosci*. 18, (Oct 2003) 1731-8, 0953-816X
- Sonntag, K. C., Simantov, R., Kim, K. S. & Isacson, O. (2004). Temporally induced Nurr1 can induce a non-neuronal dopaminergic cell type in embryonic stem cell differentiation. *Eur J Neurosci*. 19, (Mar 2004) 1141-52, 0953-816X
- Tropepe, V., Hitoshi, S., Sirard, C., Mak, T. W., Rossant, J. & van der Kooy, D. (2001). Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. *Neuron*. 30, (Apr 2001) 65-78, 0896-6273
- Verani, R., Cappuccio, I., Spinsanti, P., Gradini, R., Caruso, A., Magnotti, M. C., Motolese, M., Nicoletti, F. & Melchiorri, D. (2007). Expression of the Wnt inhibitor Dickkopf-1 is required for the induction of neural markers in mouse embryonic stem cells differentiating in response to retinoic acid. *J Neurochem*. 100, (Jan 2007) 242-50, 0022-3042
- von Bohlen und Halbach, O., Schober, A., and Krieglstein, K. (2004). Genes, proteins, and neurotoxins involved in Parkinson's disease. *Prog Neurobiol*. 73, (Jun 2004) 151-77, 0301-0082
- Wallen, A. & Perlmann, T. (2003). Transcriptional control of dopamine neuron development. *Ann N Y Acad Sci*. 991, (Jun 2003) 48-60, 0077-8923
- Watanabe, K., Kamiya, D., Nishiyama, A., Katayama, T., Nozaki, S., Kawasaki, H., Watanabe, Y., Mizuseki, K. & Sasai, Y. (2005). Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci*. 8, (Mar 2005) 288-96, 1097-6256
- Wernig, M., Benninger, F., Schmandt, T., Rade, M., Tucker, K. L., Bussow, H., Beck, H. & Brustle, O. (2004). Functional integration of embryonic stem cell-derived neurons in vivo. *J Neurosci*. 24, (Jun 2004) 5258-68, 0270-6474
- Wichterle, H., Lieberam, I., Porter, J. A. & Jessell, T. M. (2002). Directed differentiation of embryonic stem cells into motor neurons. *Cell*. 110, (Aug 2002) 385-97, 0092-8674
- Wurst, W. & Bally-Cuif, L. (2001). Neural plate patterning: upstream and downstream of the isthmus organizer. *Nat Rev Neurosci*. 2, (Feb 2001) 99-108, 1471-003X

- Yamada, T., Placzek, M., Tanaka, H., Dodd, J. & Jessell, T. M. (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell*. 64, (Feb 1991) 635-47, 0092-8674
- Ye, W., Shimamura, K., Rubenstein, J. L., Hynes, M. A. & Rosenthal, A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell*. 93, (May 1998) 755-66, 0092-8674
- Ying, Q. L., Stavridis, M., Griffiths, D., Li, M. & Smith, A. (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nat Biotechnol*. 21, (Feb 2003) 183-6, 1087-0156
- Zetterstrom, R. H., Solomin, L., Jansson, L., Hoffer, B. J., Olson, L. & Perlmann, T. (1997). Dopamine neuron agenesis in Nurr1-deficient mice. *Science*. 276, (Apr 1997) 248-50, 0036-8075
- Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O. & Thomson, J. A. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 19, (Dec 2001) 1129-33, 1087-0156

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Edited by Prof. Craig Atwood

ISBN 978-953-307-196-1

Hard cover, 672 pages

Publisher InTech

Published online 26, April, 2011

Published in print edition April, 2011

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How to reference

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Gilda Guerrero-Flores and Luis Covarrubias (2011). Dopaminergic Differentiation Potential of Neural Precursor Cells Derived from Embryonic Stem Cells, *Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis*, Prof. Craig Atwood (Ed.), ISBN: 978-953-307-196-1, InTech, Available from: <http://www.intechopen.com/books/embryonic-stem-cells-the-hormonal-regulation-of-pluripotency-and-embryogenesis/dopaminergic-differentiation-potential-of-neural-precursor-cells-derived-from-embryonic-stem-cells>

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Phone: +86-21-62489820
Fax: +86-21-62489821

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