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Chapter 7

SPECIES-SPECIFIC MOLECULAR MECHANISMS ESTABLISHING THE DOPAMINE NEURONAL PHENOTYPE

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SUMMARY

Throughout the animal kingdom, dopamine neurons are used to regulate essential behaviors ranging from locomotion to odor detection. Specification and maintenance of the dopaminergic phenotype requires the coordinated transcription of a cassette of genes that are necessary for the function of dopamine neurons. Defining the molecular mechanisms that regulate transcription of this gene cassette in humans is central to understanding nervous system development as well as the etiology and pathology of dopamine-related diseases. However, our understanding of these molecular mechanisms in humans is based largely on studies conducted in animal models. This chapter reviews reports of species-dependent variation in the regulatory mechanisms that control dopaminergic gene cassette transcription in widely used animal models. These studies are important guides for understanding the limitations of different animal models that are used to investigate human dopamine neuron development and disease.

INTRODUCTION

Dopamine neurons are found throughout the animal kingdom and are necessary for locomotor behavior as well as learning and memory (Chase and Koelle, 2007; Joshua et al., 2009; Waddell, 2010; Wise, 2004). In mammals, dopamine neurons also have well-established roles in regulating lactation, odorant detection and light/dark visual adaption (Cave and Baker, 2009; Fitzgerald and Dinan, 2008; Witkovsky, 2004). Dopamine neuron dysfunction in humans is associated with several debilitating conditions, including

Parkinson's Disease, schizophrenia, depression, drug addiction and attention deficit disorder (Iversen et al., 2010).

In the vertebrate brain, there are several distinct groups of dopamine neurons that each have unique morphologies and specific projection patterns (Smeets and Gonzalez, 2000; Yamamoto and Vernier, 2011). All dopamine neurons, however, share the expression of a "gene cassette" containing: *tyrosine hydroxylase (Th*; rate-limiting enzyme that converts tyrosine to L-DOPA), *aromatic amino acid decarboxylase (Aadc*; converts L-DOPA to dopamine), *vesicular monoamine transporter* (*Vmat*; which packages dopamine into synaptic vesicles) and *dopamine transporter* (*Dat*; for dopamine re-uptake) (Figure 1A) (Hobert et al., 2010). The specification and maintenance of the dopaminergic phenotype requires coordinated transcriptional regulation of this gene cassette.

Genetic mutations that reduce transcription of dopaminergic cassette genes can generate phenotypes that are similar to those caused by hypomorphic mutations in the corresponding protein coding regions. For example, the neurometabolic disease, Tyrosine Hydroxylase Deficiency, is caused by decreased TH enzyme activity and is characterized by the development of movement disorders within the first 5 years after birth (Willemsen et al., 2010). Treatment with L-DOPA usually produces moderate to good outcomes. Underlying Tyrosine Hydroxylase Deficiency are homozygous mutations in either the proximal promoter or the protein coding sequence of the *Th* gene (Willemsen et al., 2010). Mutations in the coding sequence impair the capacity of TH to covert tyrosine into L-DOPA. By contrast, pathological mutations in the promoter region occur in a cyclic AMP response element (CRE), previously shown to be necessary for *Th* transcription in rodents (Huang et al., 1991; Kim et al., 1993; Lazaroff et al., 1995; Lewis et al., 1987; Trocme et al., 1998). The mutations in the promoter region presumably reduce *Th* transcription so that catecholamine biosynthesis is reduced.

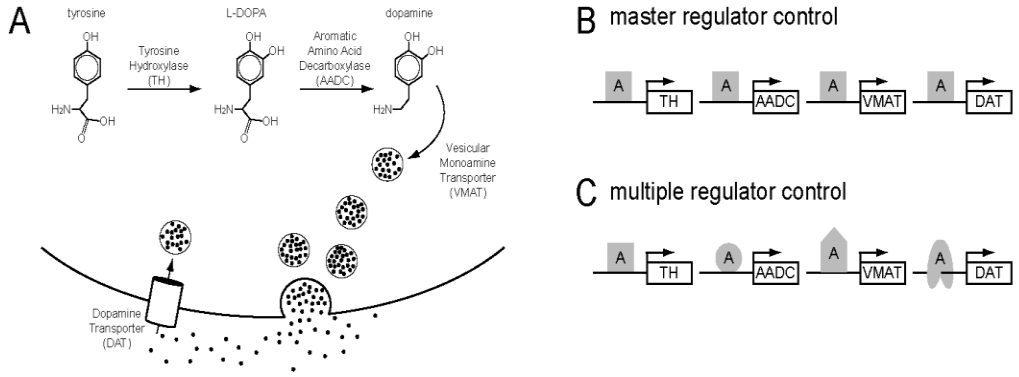

Figure 1. Dopamine gene cassette function and regulation. A. Function of proteins encoded by the dopaminergic gene cassette. Tyrosine hydroxylase (TH) converts tyrosine to L-DOPA and is the rate-limiting enzyme in dopamine biosynthesis. Aromatic amino acid decarboxylase (AADC) converts L-DOPA to dopamine. Vesicular monoamine transporter (VMAT) packages dopamine into synaptic vesicles) and Dopamine Transporter (DAT) mediates dopamine re-uptake. B and C, alternative mechanisms for coordinating the coexpression of dopaminergic cassette gene expression. B, all cassette genes are co-regulated by the same set of transcription factor activators (labeled "A") or, C, each gene is regulated independently by different sets of transcription factor activators (labeled "A").

Understanding the molecular mechanisms that underlie specification of the human dopaminergic phenotype, as well as the etiology and pathology of human dopaminergic neuron-related diseases, requires the use of animal models. Information gleaned from these models, however, will impact human health only if the mechanisms are conserved in humans. This review focuses on reports of species-dependent variation in molecular mechanisms that regulate transcription of the dopaminergic gene cassette in widely used animal models. These studies are important guides for understanding the limitations inherent to different animal models in elucidating both specification of the human dopaminergic phenotype and the etiology of dopamine-related human diseases.

MASTER REGULATORS VS. COMBINATORIAL CODES

Establishment of the dopaminergic phenotype requires co-expression of the cassette genes defined above. A fundamental question is how transcription of these genes is coordinated. Two possible mechanistic alternatives have been proposed to achieve this coordination: 1, all cassette genes are co-regulated by the same set of transcription factors (Figure 1B); or 2, each gene is regulated independently by different sets of transcription factors (Figure 1C) (Flames and Hobert, 2009). These models can be experimentally tested by examining the *cis*-regulatory regions that activate transcription of each cassette gene in dopaminergic neurons. In the first model, the regulatory regions for each gene contain a shared motif that is bound by the same transcription factor or family of transcription factors. Alternatively, in the second model, the nucleotide sequence of each regulatory region is unique and targeted by different transcription factors.

To establish whether a common set of transcription factors controls expression of the dopaminergic cassette genes in *C.elegans* (*i.e*. the first model), proximal promoter regions were analyzed for shared cis-regulatory elements (Flames and Hobert, 2009). These studies identified a short nucleotide sequence (named the "dopamine (DA)-motif") that contained an apparent binding site for ETS-domain transcription factors. A parallel and extensive set of genetic experiments showed that *axon steering defective* (*ast-1*), which encodes an ETS-domain transcription factor, was necessary and sufficient for expression of gene cassette members (Flames and Hobert, 2009). Together, these findings indicated that *ast-1* functions in *C.elegans* as a "terminal selector gene" that is required for transcription of the dopaminergic cassette by binding the DA-motif.

To establish that ETS-domain transcription factors were also necessary for specification of vertebrate dopaminergic neurons, the authors also showed that there was a reduced number of TH-expressing neurons in the olfactory bulb (OB) of mice deficient in the ETS-domain transcription factor ER81 (ETV1)(Flames and Hobert, 2009). They also reported that the DA-motif identified in nematodes was near the transcription start sites of dopaminergic cassette genes in mice and humans. Mutation of the DA-motif in the mouse *Th* proximal promoter blocked activation of reporter gene transcription mediated by ER81 in cultured cells. These findings showed that the function of *Er81* in the mammalian OB was orthologous to that of *ast-1* in *C.elegans*, suggesting that ETS-domain transcription factors and the DA-motif are an evolutionary conserved regulatory logic that programs the dopaminergic phenotype (Flames and Hobert, 2009).

A more extensive examination of mammalian dopaminergic genes, however, challenged the conclusion that the ETS-domain/DA-motif regulatory logic is conserved in mammals. In cows and dogs, the proximal promoters of dopaminergic cassette genes display poor conservation of the DA-motifs (Cave et al., 2010). Consistent with this observation, chromatin immunoprecipitation (ChIP) assays with canine olfactory bulb tissue did not detect ER81 binding to the promoter regions of dopaminergic cassette genes. Furthermore, studies in *Er81* mutant mice showed that expression of *Aadc*, *Vmat2* and *Dat* were not reduced in the OB, indicating that ER81 was not necessary for their expression in this brain region. Additionally, analysis of other ETS-domain proteins indicated that other members of this protein family do not substitute for ER81 (Cave et al., 2010). Together, these findings indicated that there are fundamental differences in the mechanisms by which ETS-domain transcription factors specify the dopaminergic phenotype in nematodes and in the mammalian OB.

The studies in the *Er81* mutant mice, however, did confirm that *Er81* was important for TH expression in a majority of mouse OB dopaminergic neurons (Cave et al., 2010; Flames and Hobert, 2009). ER81 was found to directly bind the murine *Th* proximal promoter at a site that overlaps the DA-motif, but ER81 binding to the equivalent site in the human *Th* promoter was not observed (Cave et al., 2010). Thus, the specification of the mammalian OB dopaminergic phenotype by ER81 directly targeting *Th* transcription is a rodent-specific mechanism (Cave et al., 2010).

In mammalian midbrain dopaminergic neurons, the ETS-domain transcription factor ERM (ETV5) was predicted to function as a terminal selector (Flames and Hobert, 2009). However, midbrain ERM expression was not found until post-natal day 7, which is long after terminal differentiation of these neurons is completed during mid-gestation (Wang and Turner, 2010). Furthermore, $Erm^{-/-}$ mice showed no change in either the midbrain expression of TH and DAT or the striatal innervation by TH-immunoreactive fibers originating in the midbrain. In conjunction with an examination of mRNA expression patterns for several other ETS-domain transcription factors, these findings indicated that ETS-domain proteins were not required for either initiation or maintenance of the midbrain dopaminergic phenotype (Wang and Turner, 2010). Together, the studies with ER81 and ERM strongly suggest that ETS-domain transcription factors do not function as master regulators of the mammalian dopaminergic phenotype.

Specification of dopaminergic neurons in the *Drosophila melanogaster* embryonic ventral nerve cord is also not consistent with a role for a master regulator. The LIM-homeodomain transcription factor TAILUP (TUP/ISLET) is required for the expression of TH, AADC and DAT (Stagg et al., 2011; Thor and Thomas, 1997). TUP expression, however, is not limited to the dopaminergic phenotype since it is also required for specification of serotonergic neurons. This dual role in specifying both dopaminergic and serotonergic phenotypes is consistent with the function of AADC in biosynthesis of both neurotransmitters. Recent reports have shown TUP is not sufficient for the dopaminergic phenotype and that the basic-helix-loop-helix (bHLH) transcription factor LETHAL OF SCUTE (L'SC) is also necessary for the expression of TH, AADC and DAT (Stagg et al., 2011). Thus, specification of the dopaminergic phenotype in the *Drosophila* embryonic ventral nerve cord differs from both the nematode and vertebrate and requires the binary combinational expression of, at least, L'SC and TUP.

Combinatorial codes of transcription factors have been proposed to specify the large number of distinct neuronal phenotypes in the vertebrate the spinal cord, cortex and olfactory bulb (Allen et al., 2007; Brill et al., 2008; Gelman and Marin, 2010; Guillemot, 2007; Jessell, 2000; Shirasaki and Pfaff, 2002; Wonders and Anderson, 2006). One principal advantage of controlling phenotypic differentiation through combinatorial mechanisms is that even a small number of transcription factors can mediate generation of a large range of phenotypes. In organisms with relatively simple nervous systems, however, combinatorial codes may be unnecessary and expression of a single terminal selector (or "master regulator") is sufficient to establish specific neuronal phenotypes. *C.elegans* hermaphrodites, for example, have only 302 neurons, of which 8 are dopaminergic (Chase and Koelle, 2007). The finding that *ast-1* is necessary and sufficient for specifying the dopaminergic phenotype in *C.elegans* is consistent with the possibility that this factor acts as a master regulator of the nematode dopamine phenotype (Flames and Hobert, 2009). However, even in modestly more complex organisms, such as *Drosophila* larvae which have about 80 dopaminergic neurons (Monastirioti, 1999), master regulators are not sufficient and at least a binary code of transcription factors is required to activate transcription of the dopaminergic gene cassette.

DIVERGENT ROLES OF TRANSCRIPTION FACTORS IN MAMMALS AND ZEBRAFISH

Zebrafish are a powerful vertebrate model system to investigate molecular and genetic mechanisms regulating specification and terminal differentiation of dopaminergic neurons. There are several methodological advantages to zebrafish, including transparent embryos that enable direct visualization of morphogenesis and detection of in vivo fluorescent reporters. Techniques for genetic manipulation are well established and zebrafish are readily amenable for phenotype-based genetic screens. Furthermore, the zebrafish genome has been sequenced and annotated which allows for comparisons of genomic sequence and structure with mammals.

The spatial organization of dopaminergic neuron groups in the zebrafish brain is similar, but not identical, to that in mammals (Schweitzer and Driever, 2009; Smeets and Gonzalez, 2000; Yamamoto and Vernier, 2011). One major difference is that zebrafish do not have mesodiencephalic dopaminergic neurons, but the diencephalic dopaminergic neurons of the posterior tuberculum are functionally similar (Rink and Wullimann, 2001; Schweitzer and Driever, 2009). Despite this difference, zebrafish are increasingly being used as a model system to investigate mechanisms underlying degeneration of mammalian midbrain neurons during Parkinson's Disease (Bandmann and Burton, 2010; Pienaar et al., 2010).

Regional organization in the vertebrate central nervous system is established by partially overlapping expression domains of transcription factors during development (Jessell, 2000; Liu and Joyner, 2001; Ragsdale and Grove, 2001; Shirasaki and Pfaff, 2002). Thus, both regional organization and specification of neuronal phenotypes are dependent on the combinatorial expression of sets of transcription factors. This shared reliance on combinatorial transcription factor codes suggests a linkage between molecular mechanisms that establish regional organization and acquisition of specific neuronal phenotypes (Guillemot, 2007). Thus, the similarity in the general spatial organization of dopaminergic

cell groups within mammalian and zebrafish brains suggests that some of the transcription factors necessary for establishing regional identity also may control the dopaminergic phenotype within those regions.

Mammalian midbrain dopaminergic neurons are generated from progenitors in the developing mesodiencephalon that are specified by a spatial and temporal expression pattern of transcription factors that include NURR1 and PITX3 (Smidt and Burbach, 2007). In the midbrain, both NURR1 and PITX3 are co-expressed with TH and reported to directly target and cooperatively activate transcription of several genes that are critical for the midbrain dopamine phenotype, including TH, DAT and VMAT2 (Hwang et al., 2009; Jacobs et al., 2009b; Martinat et al., 2006). Consistent with this molecular mechanism, all midbrain dopaminergic neurons are eliminated in *Nurr1* loss-of-function mutations and dopaminergic neurons in the substantia nigra pars compacta are specifically lost when *Pitx3* is mutated (Hwang et al., 2003; Nunes et al., 2003; Saucedo-Cardenas et al., 1998; Smidt et al., 2004; Zetterstrom et al., 1997).

In contrast to mammals, the orthologous zebrafish *Nurr1* and *Pitx3* genes do not have identical roles in the terminal differentiation of the dopamine phenotype. In the developing zebrafish diencephalon, *Pitx3* is not co-expressed with TH and the *Nurr1* homologues have only very limited co-expression with TH (Blin et al., 2008; Filippi et al., 2007). Furthermore, knock-down of *Nurr1* zebrafish orthologues produces only subtle, if any, changes in the number of the posterior tuberculum dopaminergic neurons (Blin et al., 2008; Filippi et al., 2007). Knock-down of *Pitx3* severely reduces the number of dopaminergic neurons, but this apoptotic cell loss is not specific to the diencephalic dopaminergic neurons and can be ameliorated by concomitant knock-down of the pro-apoptotic gene, *p53* (Filippi et al., 2007). Zebrafish express a second *Th* gene (*Th2*), but *Th2* is not expressed in the posterior tuberculum and is not an alternative target for either NURR1 or PITX3 proteins in this region (Candy and Collet, 2005; Chen et al., 2009; Filippi et al., 2010; Yamamoto et al., 2010).

The findings in zebrafish clearly indicate that the direct regulation of dopaminergic gene cassette transcription by NURR1 and PITX3 is not conserved between the mammalian mesodiencephalon and zebrafish diencephalon. However, the zebrafish studies do suggest that these transcription factors have conserved roles in the specification and survival of dopaminergic progenitors. Knock-down of zebrafish *Nurr1* paralogues increased expression of apoptotic markers in the posterior tuberculum (Blin et al., 2008). In rat midbrain dopaminergic neurons, *Nurr1* promotes survival, in part, by directly regulating transcription of *Ret*, which encodes a tyrosine receptor kinase that is an essential component of GDNF receptors (Galleguillos et al., 2010; Wallen et al., 2001). Zebrafish *Pitx3* also has an important role in the survival of diencephalic dopaminergic progenitors, as discussed above, and *Pitx3* expression in this region is controlled by the *Lmx1b* paralogues (Filippi et al., 2007). The control of zebrafish *Pitx3* expression by *Lmx1b* genes presumably underlies the reduction in the number of diencephalic dopaminergic neurons when the *Lmx1b* genes are knocked down. The zebrafish *Lmx1b* genes are also not co-expressed with *Th*, but they are co-expressed with *Sox2*, a marker of neural stem cells (Filippi et al., 2007). Together, these finding suggest that the zebrafish *Lmx1b* and *Pitx3* genes are expressed in diencephalic dopaminergic progenitors and regulate their survival. In *Lmx1b* knockout mice, *Pitx3* expression is also lost and mesencephalic dopamine neurons do not survive (Smidt et al., 2000). In contrast to the general survival role played by *Pitx3* in zebrafish, mice lacking *Pitx3* specifically lose midbrain dopaminergic neurons in the substantia nigra (Hwang et al., 2003;

Nunes et al., 2003; Smidt et al., 2004). Thus, specification and maintenance of dopaminergic progenitors is an evolutionary conserved function of NURR1, PITX3 and LMX1B. By contrast, the function of these transcription factors in the terminal differentiation of dopaminergic phenotype is limited to mammals (Figure 2A).

Foxa2 expression is another example of the evolutionary divergence in the developmental functions of regional patterning transcription factors. In mice, the FOXA2 transcription factor is expressed in the floor plate of the developing central nervous system starting around embryonic day 8 (Ang et al., 1993; Monaghan et al., 1993; Sasaki and Hogan, 1994). FOXA2 is critical for the specification of progenitors that give rise to midbrain dopaminergic neurons and hindbrain serotonergic neurons (Ang, 2009; Jacob et al., 2007) FOXA2 has been reported to directly bind the *Th* promoter and regulate its transcription in the midbrain dopaminergic progenitors, (Lee et al., 2010; Lin et al., 2009). Homozygous loss of *Foxa2* is embryonic lethal in mice and prevents floor plate formation as well as generating significant mesodermal and endodermal defects (Ang and Rossant, 1994; Hallonet et al., 2002; Weinstein et al., 1994). Conditional knockout of *Foxa2* during embryonic development eliminates NURR1 as well as a TH and AADC expression in the developing midbrain regions (Ferri et al., 2007). In addition to this developmental role, heterozygous *Foxa2* mutant mice (aged ≥18 months old) were found to have major motor abnormalities (Kittappa et al., 2007). The *Foxa2*$^{+/-}$ mice spontaneously develop motor deficits and have reduced numbers of dopaminergic neurons specifically in the substantia nigra, but normal numbers in the ventral tegmental area, when compared to age-matched wild-type controls. This region-specific neurodegeneration of midbrain dopaminergic neurons and the accompanying motor deficits have a strong resemblance to pathology of Parkinson's Disease in humans.

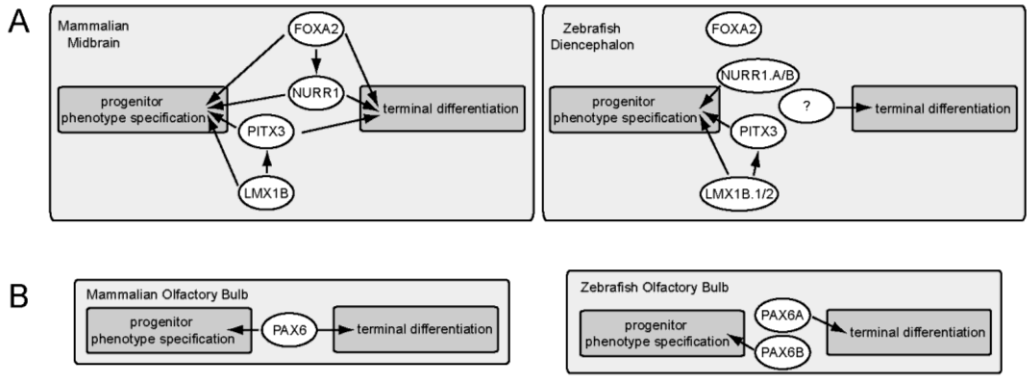

Figure 2. Evolutionarily conserved and divergent functions of transcription factors in the specification and terminal differentiation of vertebrate dopaminergic neurons. A, in the mammalian midbrain, the FOXA2, NURR1, PITX3 and LMX1B transcription factors are important for both specification of the dopaminergic phenotype as well as expression of the dopaminergic cassette genes during terminal differentiation. By contrast, the orthologous NURR1, PITX3 and LMX1B proteins in zebrafish specify dopamine progenitor phenotype and do not contribute to terminal differentiation. FOXA2 is dispensable for the dopaminergic phenotype in zebrafish. The zebrafish transcription factors necessary for dopaminergic cassette gene expression and terminal differential have not been identified (indicated by question mark). B, in the mammalian olfactory bulb, PAX6 is critical for both specification and terminal differentiation of dopaminergic neurons. In zebrafish, the progenitor specification and terminal differentiation functions performed by the single mammalian PAX6 protein appears to be divided between the homologous PAX6A and PAX6B genes.

Consistent with the function of *Foxa2* in mice, zebrafish *Foxa2* is also expressed in the embryonic floor plate (Odenthal and Nusslein-Volhard, 1998) and knockdown of *Foxa2* blocks development of serotonergic neurons in the hindbrain (Norton et al., 2005). In contrast to its function in mice, the loss of *Foxa2* does not disrupt *Th* expression in the zebrafish diencephalic dopaminergic neurons (Norton et al., 2005). The loss of *Foxa2* in zebrafish also does not prevent floor plate formation, although the floor plate does fail to expand and differentiate. Furthermore, zebrafish embryos lacking *Foxa2* also do not have the mesodermal and endodermal defects that are observed in the *Foxa2*-knockout mice (Norton et al., 2005).

Together, these findings show that similar spatial and temporal expression of transcription factor orthologues in different species does not mean that all of the regulatory functions of these proteins are conserved. Many transcription factors are pleiotropic and have multiple functions in the development of specific phenotypes, but as discussed above, some of these functions are species-specific. Understanding the developmental function of a specific transcription factor, however, can be obscured by the expression of protein homologues. Many zebrafish genes have homologues that were generated by an apparent whole genome duplication event (Taylor et al., 2001). In the case of *Foxa2*, however, maintained expression of *Foxa* and *Foxa1* homologues is dependent on *Foxa2* and the knock down of either *Foxa* or *Foxa1* has no observable phenotype (Norton et al., 2005). Also, *Nurr1* and *Lmx1b* each have two homologues in zebrafish, but none of the homologues studied were co-expressed with TH in the diencephalic dopaminergic neurons (Blin et al., 2008; Filippi et al., 2007). Thus, the differences in the function of *Foxa2*, *Nurr1* and *Lmx1b* between mammals and zebrafish are not likely a result of redundant expression of protein homologues.

The homologous PAX6 transcription factors in zebrafish, however, do have differential effects on the specification and terminal differentiation of dopaminergic neurons in the OB. Mammals have a single *Pax6* gene that encodes a paired-homeodomain transcription factor and specifies OB dopamine neurons (Allen et al., 2007; Brill et al., 2008; Dellovade et al., 1998; Hack et al., 2005; Kohwi et al., 2005). In both embryonic and adult wild-type mice, PAX6 is expressed in both neural progenitor cells and in mature OB dopaminergic neurons together with GAD67 (Dellovade et al., 1998; Kohwi et al., 2005; Parrish-Aungst et al., 2007; Stoykova and Gruss, 1994). Recent studies have shown that PAX6 is also critical for the survival of these OB neurons (Ninkovic et al., 2010). In zebrafish, there are two homologous *Pax6* genes (*Pax6a* and *Pax6b*) that have complementary expression patterns (Adolf et al., 2006). *Pax6b* expression is restricted to neural progenitors found in the telencephalic region that are capable of generating OB dopaminergic progenitors. By contrast, *Pax6a* expression is absent from the neural progenitors and is co-expressed with *Gad67* in mature OB dopaminergic neurons. Thus, progenitor specification and terminal differentiation functions performed by the single mammalian *Pax6* gene appear to be divided between the zebrafish *Pax6a* and *Pax6b* genes (Figure 2B). Mutation of homologous zebrafish *Pax6* genes individually would be expected to have phenotypes that only partially overlap with the mutations associated with mammalian *Pax6*.

Zebrafish are an important model system to investigate the specification, differentiation and degeneration of dopaminergic neurons. As the studies discussed here show, however, there are some important differences in the transcription codes and molecular mechanisms that mediate these processes. In the OB, these differences illustrate how gene homologues in zebrafish can differentially perform the functions that are otherwise carried by a single mammalian gene. In the mammalian mesodiencephalon and zebrafish diencephalon, the

differences in the terminal differentiation of the dopaminergic neurons reflects a more fundamental divergence in molecular regulatory mechanisms. However, the mechanisms that specify and maintain dopaminergic neuronal progenitors in these regions are conserved and make zebrafish a potentially powerful model system to establish mechanisms that are conserved through out vertebrates.

SPECIES-SPECIFIC FUNCTION OF DJ-1 IN MOUSE MODELS OF PARKINSON'S DISEASE

Parkinson's Disease (PD) affects several different neuronal phenotypes in the CNS (Halliday and McCann, 2010). The most well studied pathological feature is the degeneration of dopaminergic neurons in the substantia nigra. Although the majority of PD cases are sporadic (no known environmental or genetic cause), there are a growing number of genetic mutations associated with hereditary forms of PD (Lee and Liu, 2008; Lesage and Brice, 2009). Mouse models incorporating these genetic mutations are widely used for studies to establish the molecular and genetic mechanisms that underlie PD, but a surprising observation is that most of these mouse models of human genetic mutations lack neurodegeneration in the substantia nigra (Dawson et al., 2010). The causes underlying this species-specific discrepancy in PD pathology are unknown, but they are essential for understanding the limitations of mouse models for human PD.

Autosomal recessive mutations in *DJ-1* (*Park7*) underlie one of the familial forms of PD (Abou-Sleiman et al., 2003; Annesi et al., 2005; Bonifati et al., 2003; Hague et al., 2003; Hedrich et al., 2004; Hering et al., 2004; Macedo et al., 2009). The *DJ-1* gene is expressed in both astrocytes and neurons throughout the brain and it encodes a member of the THIJ/PFPL family of molecular chaperones that functions in several cellular processes, including oxidative stress, gene expression and apoptosis (Kahle et al., 2009; Lev et al., 2006). Pathogenic mutations in *DJ-1* lead to an early-onset motor dysfunction that progresses slowly and responds well to treatment with L-DOPA (Bonifati et al., 2004). Mouse *DJ-1* knock-out models have some locomotor defects, but they do not exhibit dopaminergic neurodegeneration and have normal dopamine expression levels (Andres-Mateos et al., 2007; Chandran et al., 2008; Chen et al., 2005; Goldberg et al., 2005; Kim et al., 2005; Manning-Bog et al., 2007). *DJ-1* mutant mice, however, are sensitized to oxidative stress induced by neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Goldberg et al., 2005; Kim et al., 2005; Manning-Bog et al., 2007; Taira et al., 2004). Similar to the mouse models, knockout of *DJ-1* in *C.elegans* and *Drosophila* does not produce dopaminergic neuron-specific degeneration, but does increase sensitivity to oxidative stress (reviewed in (Kahle et al., 2009). Thus, the evidence indicates that DJ-1 has an evolutionarily conserved role in mitigating oxidative stress that is not unique to dopaminergic neurons.

Recent investigations have provided insight into the human-specific function of DJ-1 as a transcription co-activator in dopaminergic neurons. In cultured human cells, RNA interference and protein over-expression experiments have shown that DJ-1 is a potent co-activator of *Th* transcription (Ishikawa et al., 2010; Zhong et al., 2006). Chromatin immunoprecipitation (ChIP) assays with both cultured human cells and human substantia nigra tissue showed that DJ-1 and the PSF protein are recruited to the *Th* promoter (Ishikawa

et al., 2010; Zhong et al., 2006). PSF (Polypyrimidine tract-binding protein-associated Splicing Factor) is a multi-functional nuclear protein that can act as a transcription co-repressor and recruit histone deacetylase complexes containing HDAC1 and SIN3A (Shav-Tal and Zipori, 2002). In the absence of DJ-1, PSF is sumoylated and represses human *Th* transcription (Ishikawa et al., 2010; Xu et al., 2005; Zhong et al., 2006). In the presence of DJ-1, *Th* transcription is enhanced by physical interactions between DJ-1 and PSF that block both sumoylation of PSF and recruitment of histone deacetylases (Ishikawa et al., 2010; Zhong et al., 2006). In murine cell lines, PSF and DJ-1 also physically interact, but DJ-1 does not enhance *Th* transcription (Ishikawa et al., 2010). ChIP and electromobility shift assays indicate that human, but not mouse, PSF/DJ-1 complexes are recruited to a region approximately 2.8kb upstream from the *Th* transcription start site (Ishikawa et al., 2010). Although these studies did not identify the DNA-binding transcription factor that recruits the PSF/DJ-1 complex to the human *Th* promoter, both NURR1 and PITX3 have been shown to physically interact with PSF and ChIP-on-chip studies with both mouse MN9D cells and E14.5 midbrain neurons have shown the 2.8kb upstream region of *Th* is enriched for PITX3 and NURR1 binding (Jacobs et al., 2009b). PSF, NURR1 and PITX3 have been proposed to form a multi-protein transcription complex that regulates transcription of several genes that are critical for the midbrain dopamine phenotype (Jacobs et al., 2009a; Jacobs et al., 2009b), and studies with DJ-1 suggest that it is a human-specific co-activator of the NURR1/PITX3/PSF complex.

The human-specific regulation of *Th* transcription by DJ-1/PSF complexes also suggests that the loss of functional DJ-1 in humans can reduce *Th* expression levels. Since DJ-1/PSF complexes may be also critical co-activators for the transcription of several dopaminergic cassette genes through interactions with NURR1 and PITX3, then the loss of DJ-1 would be predicted to have a broad and negative impact on the human midbrain dopaminergic phenotype. By contrast, since DJ-1 is not a co-activator for *Th* transcription in mice, then it may not be a co-activator of murine NURR1 and PITX3 transcription factors. This species-specific function of DJ-1 could explain why the midbrain dopaminergic phenotype is not compromised in *DJ-1* knock-out mice (Figure 3).

Further studies are required to establish that molecular and structural basis for species-selective recruitment of DJ-1 on the *Th* promoter. These studies, however, will need to consider alternative mechanisms by which DJ-1 can regulate dopaminergic gene transcription. Recent reports indicated that DJ-1 is also recruited to the human *Th* promoter by physical interactions with MTA-1 (Reddy et al., 2011). MTA-1 is bi-functional non-DNA binding transcription co-regulator that can mediate either repression by recruiting histone deacetylases or activation by interacting with RNA polymerase II (Manavathi and Kumar, 2007). In human neuroblastoma cells, MTA-1 physically interacts with DJ-1 and PITX3 to form a complex that is recruited to a region in *Th* that is ~9kb upstream from the transcription start site (Reddy et al., 2011). Thus, DJ-1 can co-activate PITX3 on at least two distinct sites in the human *Th* upstream region: a proximal site through interactions with PSF and a distal site by interacting with MTA-1. *Mta-1* knockout mice have movement disorders as well as reduced levels of both *Th* transcription and dopamine expression (Reddy et al., 2011). However, whether DJ-1 is recruited to the mouse *Th* ~9kb upstream region via interactions with MTA-1 and PITX3 remains to be established.

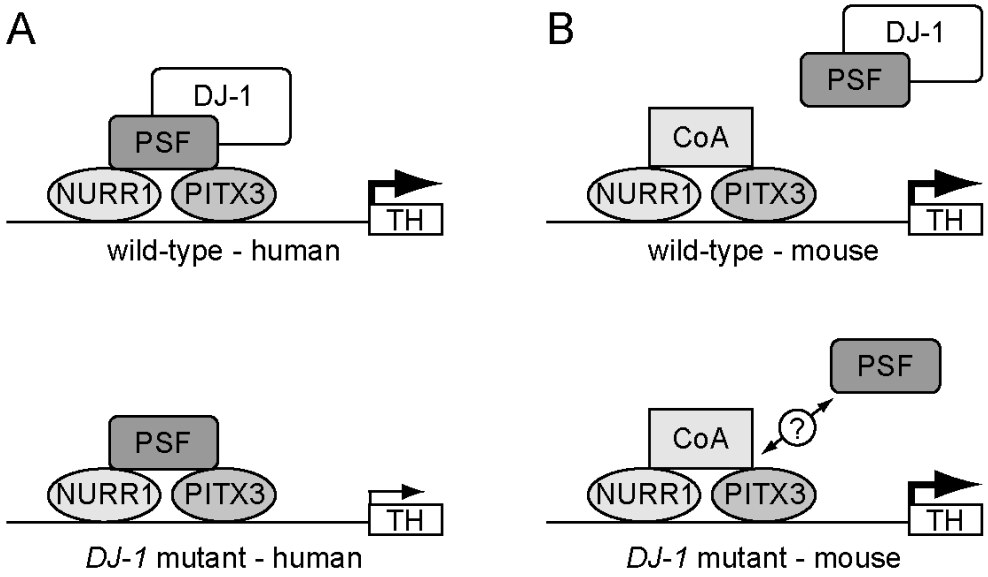

Figure 3. Model of human-specific regulation of *Th* transcription by DJ-1/PSF complexes in the midbrain. A, in wild-type human midbrain dopaminergic neurons, DJ-1 enhances *Th* transcription by co-activating PSF/NURR1/PITX3 complexes bound to the *Th* upstream region. When the *DJ-1* gene is mutated, however, *Th* transcription is reduced because the PSF/NURR1/PITX3 is not co-activated by functional DJ-1 protein. B, by contrast, in wild-type mice, NURR1/PITX3 complexes on the *Th* promoter are not co-activated by DJ-1/PSF complexes. The co-activators (CoA) recruited by the murine NURR1/PITX3 complexes are not established. Because DJ-1 is not a critical co-activator of *Th* transcription, dopamine biosynthesis is not reduced in *DJ-1* knockout mice. This human-specific co-activator function of DJ-1 at least partially explains why midbrain dopaminergic phenotype is not disturbed in *DJ-1* knockout mice, but homozygous mutation of *DJ-1* in humans produces early-onset Parkinson Disease.

Rodent transgene and gene-knockout models are a critical resource for elucidating the etiology of dopamine-related diseases, such as PD, even if they do not recapitulate all of the neuropathological features of the targeted disease. In the case of the *DJ-1*, the knockout mice will be important for establishing how the loss of DJ-1 function in mitigating oxidative stress may contribute to neurodegeneration of dopaminergic neurons as well as other neural phenotypes that are affected in PD. Thus, animal systems that only partially model a human disease can still provide important insights into the overall disease pathology. Furthermore, understanding the molecular mechanisms underlying why either vertebrate or invertebrate animal models of human dopamine-related disease do not display all of the relevant clinical features, may reveal novel neuroprotection mechanisms that stimulate development of therapeutic strategies in humans.

CONCLUSIONS

The studies presented in this chapter demonstrate that there is considerable species variation in the molecular mechanisms that regulate the co-expression of dopaminergic cassette genes in animal model systems. This variability reflects, in part, other species-

specific differences such as nervous system organization and patterning as well as genomic organization and structure. For invertebrate organisms with few dopamine neurons, such as nematodes, a single transcription factor may be sufficient for terminal differentiation of the dopaminergic phenotype. In vertebrates, however, regulation of dopaminergic gene cassette expression requires a combinatorial code of transcription factors. Comparison of dopaminergic neurons in the mammalian mesencephalon and the zebrafish diencephalon indicate that the combinatorial code of transcription factors that activate transcription of the dopaminergic cassette genes is not identical. However, the complete combinatorial code of transcription factors necessary for regulating the expression of the cassette genes remains to be established, and it is possible that there is a subset of transcription factors that are conserved between mammals and zebrafish. The extent of species-related differences in vertebrates may also be regionally dependent. In contrast to the mammalian mesencephalon/zebrafish diencephalon, many of the transcription factors necessary for specification of the dopaminergic neurons in the mammalian OB also appear to be required in zebrafish (Adolf et al., 2006). A key challenge for future studies is to establish whether there is pan-regional combinatorial code of transcription factors or transcription factor families that control dopaminergic gene cassette expression in vertebrates.

In addition to elucidating the mechanisms that coordinate co-expression, another important direction for future studies is to identify the mechanisms responsible for the differential expression of dopaminergic cassette genes in vertebrate nervous systems (Bjorklund and Dunnett, 2007; Weihe et al., 2006; Yamamoto et al., 2011). For example, both zebrafish and monkey OB dopaminergic neurons co-express TH, AADC and DAT, but have either very low or undetectable expression levels of VMAT (Weihe et al., 2006; Yamamoto et al., 2011). These OB neurons have been described as "non-exocytotic" dopaminergic neurons that either release dopamine by mechanisms other than the traditional VMAT-mediated synaptic vessel (Weihe et al., 2006) or have very high turn-over rates for the transporter which preclude VMAT detection (Yamamoto et al., 2010). An alternative example of partial dopaminergic gene cassette expression includes several rodent and primate "dopa-ergic" neuronal groups that express TH, but not AADC or VMAT (Bjorklund and Dunnett, 2007). In the rodent hypothalamus, dopa-ergic neurons have been suggested to supply L-DOPA and enable dopamine production in other neurons that express AADC, but not TH (Mel'nikova et al., 2005; Ugryumov et al., 2002).

Both vertebrate and invertebrate animal model systems will continue to be necessary for elucidating the molecular mechanisms underlying the specification, maintenance and degeneration of dopaminergic neurons. However, the inability of animal model systems to completely recapitulate both the clinical and neuropathological features of dopaminergic diseases has restrained development of novel treatment strategies for these diseases (Ahmed, 2010; Bari and Robbins, 2011; Meredith et al., 2008; Sawa, 2009). In the case of *DJ-1* knockout mice, recent studies suggest that human-specific regulation of dopaminergic gene transcription by DJ-1 underlies the inability of these knockout mice to model the selective dopaminergic degeneration of PD. The conserved role of DJ-1 in mitigating oxidative stress, however, indicates that the *DJ-1* knockout mice are likely to be a critical resource for understanding the role that oxidative stress may have in dopamine-related diseases such as PD. Thus, elucidating the molecular mechanisms underlying species-dependent variation in the either development, maintenance or degeneration of dopaminergic neurons is critical for

both understanding the nature of specific animal model system limitations and developing alternative model systems.

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