

A review on pivotal genes in dopaminergic neurons life
And its effects on Parkinson's disease

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Summary

Parkinson's disease is a neurodegenerative disorder. Dopaminergic neurons in the midbrain selectively degenerate during the course of the disease. This group of neurons are located in three distinct nuclei in the midbrain; substantia nigra pars compacta(SNpc), ventral tegmental area(VTA) and retrorubral field. The development of these three nuclei is orchestrated by the expression of a set of transcription factors. Substantia nigra pars compacta, Ventral tegmental area and retrorubral field have important roles in the control of movement and the and are involved in motivation, orgasm, cognition, drug addiction and psychiatric disorders.

The specific groups of genes which are pivotal to development of midbrain dopaminergic neurons are: Otx2, Gbx2, Pax2, En1, En2, Wnt1, Fgf8, Wnt5a, Foxa1 and Foxa2, SHH, Lmx1a and Lmx1b. These genes are essential in regional identity, fate determination, neuronal differentiation, postmitotic development and axon guidance of midbrain dopaminergic neurons. Several of the transcription factors also play a role in the survival and maintenance of the midbrain dopaminergic neurons throughout life.

Mutations or disorder in the expression of these genes would result in deficiency in the dopaminergic systems and/or mimicking the Parkinson's disease-like symptoms in the animal models of the disease. A better understanding of the functional role of the developmental transcription factors may result in a deeper understanding of the physiology of the midbrain dopaminergic neurons and in development of novel gene therapeutic agents to to halt the process of neurodegeneration. The developmental transcription factors can also be utilized for achieving optimized in vitro protocols for differentiation of embryonic stem cells into midbrain dopaminergic neurons to be used for cell replacement therapy in Parkinson's disease.

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Introduction

Neurons have different shapes, structure and functions. All neurons possess neurotransmitter phenotypes, such as dopaminergic, cholinergic or gabaergic, which define their functions. The neurons which use dopamine as neurotransmitter are known as dopaminergic neurons. In mammals majority of these neurons are located in three distinct nuclei in the midbrain;(Alavian, et al., 2008)

Substantia nigra, which has a key role in movement and reward, means black substance in Latin and seems darker than neighboring areas. This nucleus has two parts with different functions and connections;1) Substantia nigra pars compacta(SNpc) mainly works as input to the basal ganglia circuit and supplies striatum with dopamine. 2) **substantia nigra pars reticulata** that mainly serves as an output for conveying signals from basal ganglia to other brain structures.

Ventral tegmental area (VTA) is another part of midbrain nucleus located near to the midline on the floor of midbrain. VTA has a role in cognition, motivation, orgasm, drug addiction, psychiatric disorders and intense emotions related to love. The VTA connects to prefrontal cortex, the caudal brainstem and the regions in between.

Retrorubral field is an **area caudal and lateral** to red nucleus in the **midbrainreticular** formation and known as Nissal stain in the human.

Parkinson's disease is a neurodegenerative disorder characterized by the gradual loss of the midbrain dopaminergic neurons in the SNpc and a distinct set of clinical symptoms including tremor, rigidity, slowness of movement and postural instability. PD is idiopathic but genetic and environmental risk factors have been discovered. The suspected environmental risk factors include **paraquat, maneb, dieldrin, pyrethroid and organophosphates**(Baltazar, 3 February 2014). Country sides, farms and village have more PD cases, maybe because of contaminated water sources

with the pesticides. Wound and injuries of head can also be the cause of Parkinson's disease. In addition alcohol usage and smoking tobacco are imminent boosters of this disease{Noyce, 2012 #2342}. Also mutations in certain genes, like alpha-synuclein, parkin, leusine rich reapept kinase 2, PTEN-induced putative kinase 1, DJ-1 and ATP13A2, are associated with PD.

In this work I will focus on the role of the transcription factors involved in the early, intermediate and late development of the midbrain dopaminergic neurons. Several of these factors are known as survival factors that protect neurons against the environmental and genetic risk factors. The new evidence from genetic association studies also suggests a direct link between these essential genes, Nurr1, FGF8, Lmx1a, Lmx1b, En1, En2, Pax gene, Otx2, Gbx2, Wnt1, SHH and Pitx3 (figure1), and the etiology of PD(Alavian, et al., 2008; Prakash and Wurst, 2004).

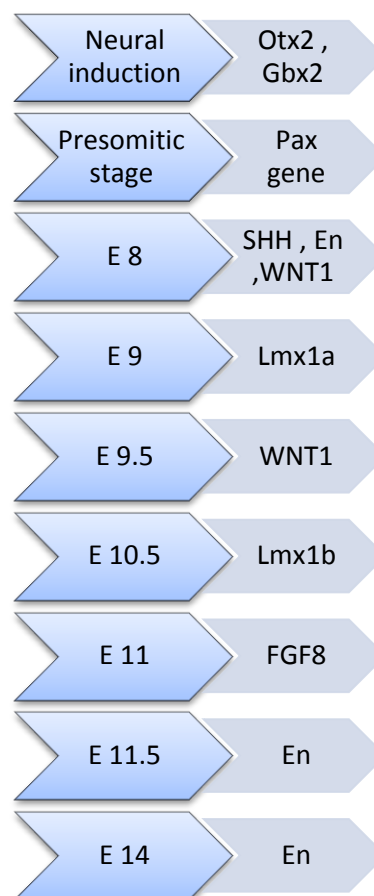


Figure1. Timing of essential genes for formation of midbrain

The developmental studies have revealed a susceptibility of the SNpc neurons for degeneration that is rooted in the transcriptional profile of these neurons. This vulnerability to degeneration is exposed through several factors including aging. Although there is not a certain age for PD, most cases occur after the age 60, although there are some early onsets. First signs of Parkinson's disease are tremor, stiffness, slowness, impaired balance and later on a shuffling gait. In addition, side effects of Parkinson's disease are depression, anxiety, and dementia and sleep disorders(Comella, 2008; Modugno, et al.). Since now there is no certain way or certain test for diagnosis. Finding Lewy bodies in midbrain in autopsy usually shows that the person had PD. Here is a summary of pathogenesis, symptoms and genetic disorders in PD:

Pathogenesis:

Cell death in the substantia nigra and the ventral section of pars compata are main pathological characteristics of Parkinson's disease. By the time of diagnosis, this region of the brain has lost 50–70% of its neurons compared with the same region in unaffected individuals {Davie, 2008 #2346}.

Immunohistochemical studies on the brainstem sections reveal macroscopic changes. Reduction of neuromelanin pigmentation in locus coeruleus and substantia nigra indicates neuronal loss in those areas{E, 2007 #2347}. Additionally, the histopathology of the substantia nigra reveals neuronal loss and the presence of Lewy bodies in many of the remaining neurons. Neuronal loss is accompanied by the death of astrocytes and activation of microglia {E, 2007 #2347}.

Basal ganglia is strongly interconnected with cerebral cortex, thalamus and brainstem and several other brain areas. The basal ganglia have an important role in a lot of functions such as; procedural learning, routine behaviors such as eye movement, cognition and emotion.{Weyhenmeyer, 2007 #2348} There are five major pathways which basal ganglia are responsible for: the motor, oculo-motor, associative, limbic and orbitofrontal circuits(Jankovic, 2008). Since these functions

are involved in voluntary movement, learning and attention, etc. disruption in these pathways explains many symptoms of PD.(Jankovic, 2008).

In the late stages of Parkinson's disease, all areas in the basal ganglia are affected by the disease. As these areas involve in wide variety functions such as attention, learning and movement, disturbance in their health conclude PD's symptoms (Jankovic, 2008).

For describing the major effects of motor circuits and its alternation with Parkinson, a conceptual model has been developed since 1980s. In this model, the basal ganglia normally perform a constant inhibitory influence on a wide range of motor systems, preventing them from becoming active at improper times. When a decision is made to exert a specific action, inhibition is reduced for the required motor system so releasing it for activation. Dopamine acts to comfort this release of inhibition, so major levels of dopamine function tend to promote motor activity, while low levels of dopamine function, which occur in PD, charge greater exertions of effort for any given movement (Jankovic, 2008).

Symptoms:

Parkinson's disease has both motor and non-motor symptoms. Non-motor symptoms include sensory and sleep difficulties, neuropsychiatric and autonomic dysfunction such as mood, cognition, behavior or thought alterations (Jankovic, 2008). The motor symptoms include tremor, rigidity, slowness of movement, and postural instability (Jankovic, 2008). The most obvious and well-known symptom of PD is Tremor. Frequency of this tremor is between 4 and 6 hertz (cycles per second). Although tremor is not problematic in around 30% of PD patients in the onset of disease but it still is the most common symptom (Jankovic, 2008). At the onset of the disease, resting tremor only affects one single arm or leg. But it gradually becomes bilateral and affects to a greater extent the most distal part of the limb (Jankovic, 2008).

Slowness of movement or bradykinesia is another symptom of PD. It makes difficulties along movement process, from planning to initiation and finally execution of a movement (Jankovic, 2008). Commonly in the early stages of the disease

Bradykinesia is a very disabling symptom especially when performing daily tasks such as writing or getting dressed {A, 2004 #2352}(Jankovic, 2008). Bradykinesia is not present in performing all tasks. For example, some people are barely able to walk but they can still ride a bicycle.

Another motor symptom of PD is rigidity. When excessive and continuous contractions increase muscle tone, the stiffness and resistance to limb movement happens (Jankovic, 2008). Tremor and increased tone together can signal the start of rigidity {E, 2007 #2354}. Rigidity may be associated with joint pain (Jankovic, 2008). Rigidity is commonly asymmetrical in early stages of Parkinson's disease. But then it tends to affect the neck and shoulder muscles and then the muscles of the face and extremities {O'Sullivan, 2007 #2355}. Rigidity typically affects the whole body and reduces the ability to move.

In the late stages of the disease one typical symptom is postural instability, leading to impaired balance and frequent falls {L, 2013 #2356} and to bone fractures (Jankovic, 2008). Instability is often not seen in the initial stages, especially in younger individuals {A, 2004 #2352}. More than 40% may experience falls and about 10% of patients may have falls weekly, number of falls is related to the intensity of PD (Jankovic, 2008).

Other recognized motor signs and symptoms include gait and posture disturbances, festination or the rapid shuffling steps and a forward-flexed posture when walking (Jankovic, 2008), Speech and swallowing disturbances including voice disorders,{J, 2012 #2357} and mask-like face expression.

Mutations:

Mutations in specific genes have been associated to Parkinson's disease. Those specific genes code for alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 {Davie, 2008 #2346}{S, 2009 #2358}. Among these genes SNCA and LRRK2 have been characterized in more detail than the other genes in relation with PD.

SNCA has received extra attention because alpha-synuclein protein is the main component of Lewy bodies {S, 2009 #2358}. Missense mutations or duplications and triplications of the locus containing it have been found in families with PD. Missense mutations, however, are rare. In stark contrast, multiplications of the SNCA locus account for about 2% of familial cases. Multiplications have been found in asymptomatic carriers, which show that penetrance is incomplete or age-dependent {S, 2009 #2358}.

Dardarin is a protein which is coded by LRRK2 gene (PARK8). LRRK2 mutations are the most common known cause of familial and sporadic Parkinson's disease. Approximately 5% of people with a family history of the PD and 3% of sporadic cases carry mutations in LRRK2 {S, 2009 #2358}{Davie, 2008 #2346}.

Embryogenesis

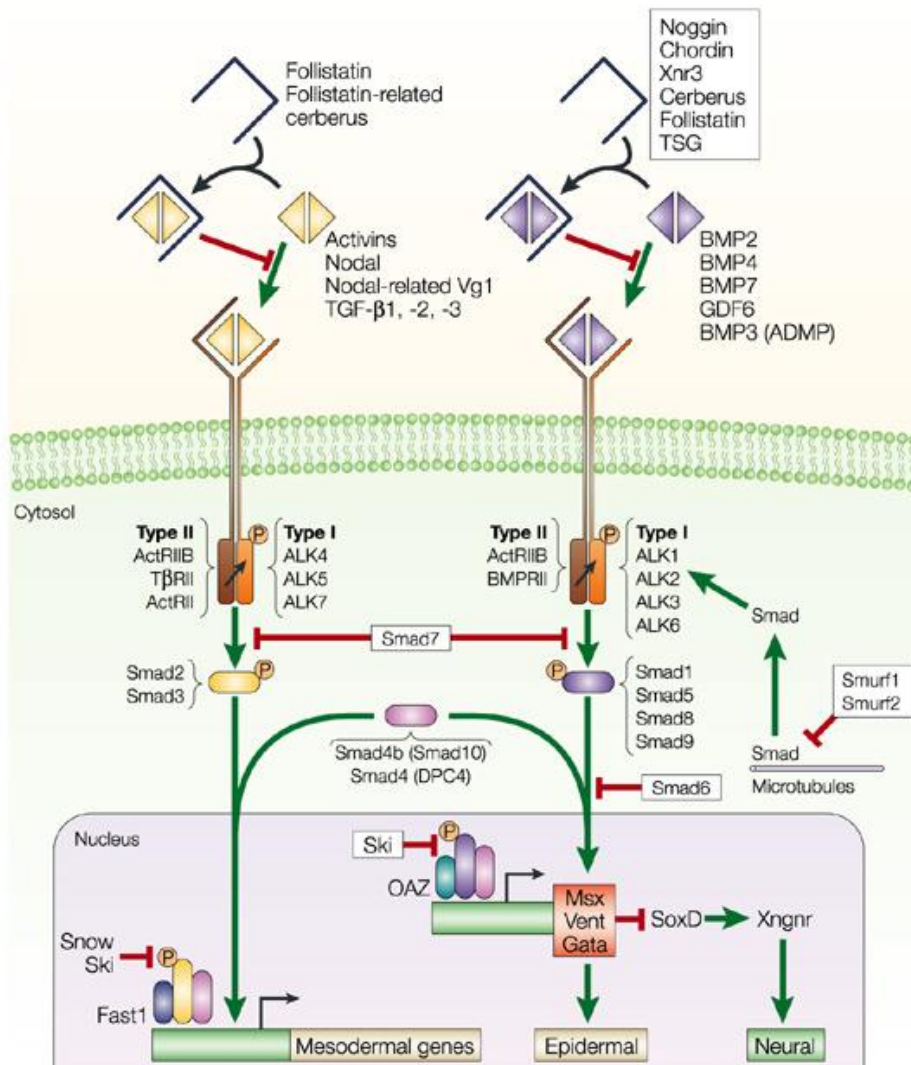
Fertilization is a fusion of gametes which produces zygote. An asymmetric zygote cell has two different parts; *animal pole* and *vegetal pole*. Animal pole will develop into ectoderm and mesoderm and vegetal pole will form endoderm. After the 7th cleavage and production of 128 cells, the embryo is shaped like spherical layers of cells and is called blastula. Blastula contains a yolk-filled cavity named blastocoel. After blastulation and before gastrulation a structure would form which is known as trophoblast. The trophoblast will form the extra embryonic tissue. 

Gastrulation is a basic and essential phase of animal embryogenesis. In this phase germ layers are specified, rearranged, and shaped into a body plan with organ fundamental elements. During **emboly**, cells of mesoderm and endoderm layers become internalized beneath the ectoderm. During gastrulation a hole on the surface of the blastula invaginates to form the dorsal lip of the blastopore. When this lip has been established, the invaginating cells will migrate along the inner wall of the blastula or the roof of blastocoel. At the same time convergent extension will occur. During this process those cells which have approached the lip intercalate

mediolaterally are pulled over the lip and inside the embryo {Gilbert, 2010 #2359}. The embryonic ectoderm would derive from the inner cell mass and it would form the nerve system {Gilbert, 2010 #2359}. The process of formation of nerve system starts with formation of thick and flat neural plate. The neural plate will fold and form the neural tube, which will pass the differentiation phase and form the brain and the spinal cord {Kenyon, 2016 #2360}. It has been shown that the primitive ectoderm in the mouse embryo is formed from inner cell mass between embryonic days 4 – 5.5 and it is implanted around the same time {Nass, 2008 #2361}.

Neurogenesis

Generation of neurons from neural stem cells is called neurogenesis. Pluripotent stem cells need guiders to differentiate. Inducers and signals control fate of neurons. Noggin which (NOG) is an inhibitory protein, encoded by the NOG gene. It inhibits TGF-beta signal transduction by binding to TGF-beta ligands and inhibiting them from binding to their receptors. In addition, noggin and chordin and follistatin have a key role in inhibition of the BMP signaling pathway (Figure 2). BMP is important for choosing the neural fate and has a signaling function in induction of epidermal fate. Noggin also has a role in bone development and joint formation {I, 2002 #2362}.



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Figure 2.BMP signaling. Green arrows indicate activation of the TGF-β pathway, red bars indicate inhibition and white boxes indicate neural inducers.

Bone morphogenetic proteins or BMPs are a group of growth factors. They are also known as Cytokines and metabologenes (Reddi and Reddi, 2009). The most important and original function of BMPs is their role in induction of bone and cartilage cells. Also BMPs are involved in a group with pivotal function as morphogenic signals. Morphogenic signals orchestrate formation of tissue and architecture throughout the body {SA, 2007 #2364}. Misregulation in bone morphogenetic protein signaling is a major cause of cancers. For example, Colon cancer is the result of BMP signaling misregulation(Kodach, et al., 2008). On the other hand, overactivation

of BMP signaling would cause other disorders such as Barretts' esophagus and it thus instrumental in the development of adenocarcinoma in the proximal portion of the gastrointestinal tract (Milano, et al., 2007).

BMP1 is a metalloprotease and BMP2 through BMP7 are members of transforming growth factor (TGF) beta superfamily (Table-1).

Table 1.BMP types (Even, et al.) All known type of BMPs and their functions.

BMP	FUNCTION
BMP1	BMP1 does not belong to the TGF- β family of proteins. It is a metalloprotease that acts on procollagen I, II, and III. It is involved in cartilage development.
BMP2	Acts as a disulfide-linked homodimer and induces bone and cartilage formation. It is a candidate as a retinoid mediator. Plays a key role in osteoblast differentiation.
BMP3	Induce bone formation
BMP4	Regulates the formation of teeth, limbs and bone from mesoderm. It also plays a role in fracture repair, epidermis formation, dorsal-ventral axis formation, and ovarian follicular development.
BMP5	Performs functions in cartilage development.
BMP6	Plays a role in joint integrity in adults. Controls iron homeostasis via regulation of hepcidin
BMP7	Plays a key role in osteoblast differentiation. It also induces the production of SMAD1. Also key in renal development and repair.
BMP8a	Involved in bone and cartilage development.
BMP8b	Expressed in the hippocampus.
BMP10	May play a role in the trabeculation of the embryonic heart.
BMP15	May play a role in oocyte and follicular development.

Inhibition of BMP signaling has a critical role in induction of neural fate. The inhibitory mechanisms involved in neurogenesis are ancient and have been conserved from arthropods to vertebrates (figure 3c) [1, 2002 #2362]. Several inducers of neural fate, including Smad7, Smurf1, Smurf2, Ski, induce their action via inhibition of the BMP signaling. At blastula-stage ectodermal cell can choose any fates. Activation of the **bone morphogenetic protein (BMP)** pathway in the ectoderm leads to the epidermal fates, while inhibition of BMP signaling induces neural fates. (Figure 3a). However, that kind of signals which mediated by the nodal/activin branch of the transforming growth factor beta (TGF-beta) pathway induces mesodermal gene expression in ectodermal cells (Munoz-Sanjuan and A, 2001).

Activation of the BMP/GDF pathway would be the cause of phosphorylation of the signal transducers Smad1, -5, -8 and -9. They form a complex with Smad4 (DPC4) or Smad4b (Smad10). This complex translocate to the nucleus. Then they associate with various developmentally regulated transcription factors, such as OAZ (Massague and Chen, 2000), to direct epidermal gene expression. The acquisition of neural fates occurs through inhibition of BMP activity (Prakash and Wurst, 2004).

Figure 1. BMP signalling and the specification of ectodermal fate.

Models proposed for the role of **bone morphogenetic protein (BMP)** signalling in the specification of ectodermal fate in frog embryos (a), chick embryos (b) and mouse embryonic stem (ES) cells (c), based on gain- and loss-of-function studies. Overall, the abrogation of BMP signals is central to the acquisition of neural fates across species. a) In amphibians, this is thought to occur mostly through the clearance of BMP ligands by BMP inhibitors secreted from the frog organizer. b) In chick embryos, BMP inhibition takes place initially through the transcriptional downregulation of BMP messages from the prospective neural territory. Fibroblast growth factor (FGF) and Wnt signals have been shown to mediate this transcriptional regulation. FGFs have been proposed to promote neural fates independently of BMP inhibition (Wilson, et al., 2001), although the exact mechanism of action and its requirement for neural induction is unclear (question mark). This model is applicable only in late-blastula/early-gastrula chick embryos, as late-gastrula epiblast is not competent to respond to these signals. c) | Recently, BMPs have been shown to regulate ectodermal fate decisions in ES cells. Viable mouse ES cells that are grown in the absence of cell-cell contacts, serum or feeder cells (Lamb and Harland, 1995) mostly adopt a neural fate. In the

presence of BMPs, the cells retain their stem-like nature. By contrast, isolated mouse ES cells that are grown on stromal feeder cells become neural (Kawasaki, et al., 2000). In this system, exposure to BMPs promotes epidermal fates, as in amphibians.

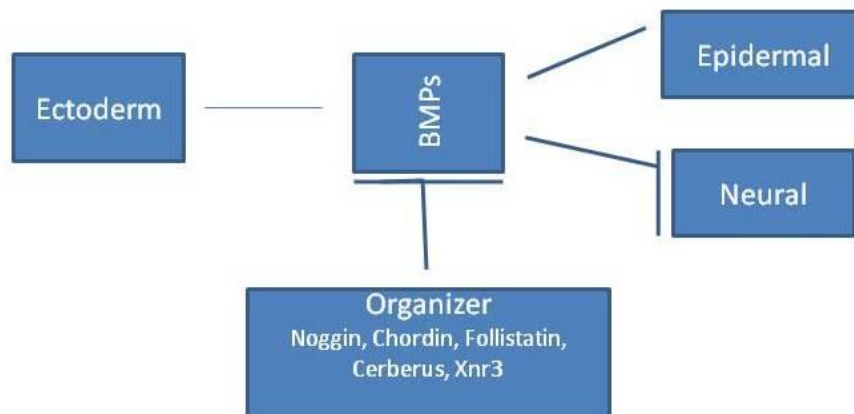


Figure3a. *Xenopus laevis*

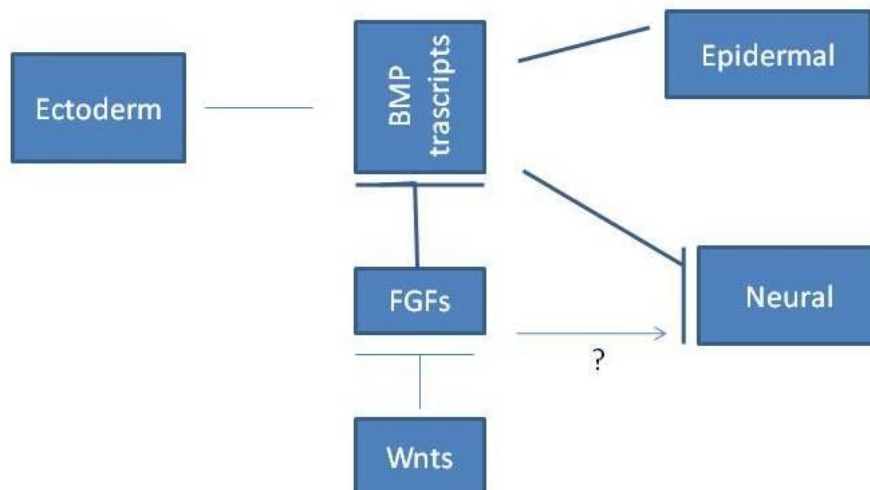


Figure3b. Chick embryos

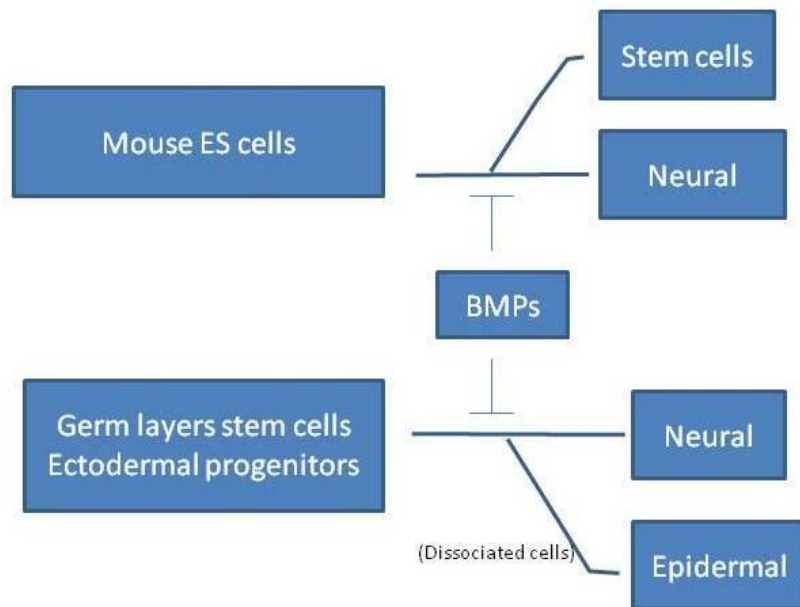


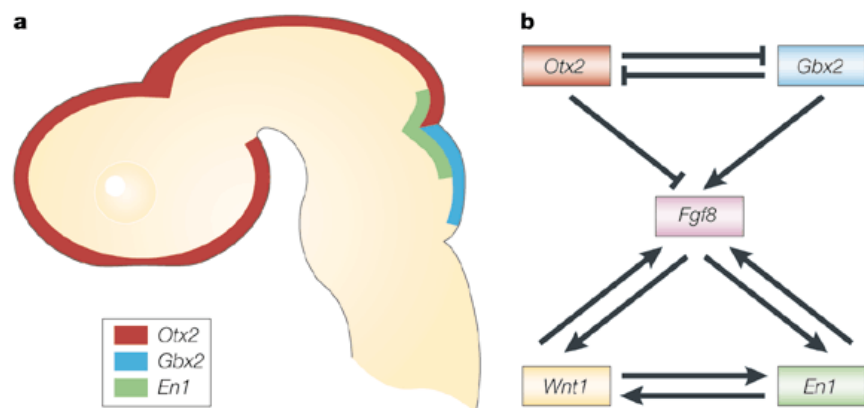
Figure3c. Mouse embryonic stem cells

Development of the midbrain and the dopaminergic neurons

Otx2 and Gbx2 are prominent for correct positioning

Ortodenticle homeobox 2 (Otx2) belongs to a paired class, bicoid subclass family of homeodomain genes and was first found in drosophila. Gastrulation brain homeobox2 (Gbx2) belongs to ANTP class, HOXL subclass homeobox. After neural induction, Otx2 is expressed in the anterior and Gbx2 in the posterior part of the neuroectoderm. At the end of gastrulation the expression domains of Otx2 and Gbx2 expand on the anterior and posterior borders, respectively, and approach each other on what will be the midbrain/hindbrain boundary (MHB). Their expressions starts at the onset of somitogenesis and transiently coexpressed in a narrow field at 4-6 somite stage (Prakash and Wurst, 2004).

Otx2 and Gbx2 are important for correct regionalization of midbrain and their expression is detectable from day 8 of gestation. Otx2 defines the forebrain and midbrain and is expressed in regions of the developing olfactory, auricular and ocular system, including the covering of the optic nerve. Gbx2 defines the hindbrain region and spinal cord. Other genes overlap expression domains with Otx2 and Gbx2. First of all Pax2, one of the paired-box transcription factors, initiated in the presomic head-fold stage around the Otx2/Gbx2 boundary (Rowitch and McMahon, 1995). Expression of glycoprotein Wnt1 and engrailed 1(En1) as a homeodomain transcription factor, initiate within Pax2 expression. En1 and Wnt1 are initially coexpressed across the Otx2/Gbx2 border in the prospective midbrain and hindbrain but, later, Wnt1 expression is largely confined to the posterior Otx2-positive domain (Prakash and Wurst, 2004). (Figure 4)



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Figure 2. Interaction among Otx2, Gbx2, En1, Wnt1 and Fgf8 effects on formation of the mid-/hindbrain region.

a. Formation of the mid-/hindbrain region is dependent on proper functioning of the isthmus organizer, which is formed by the interaction, or reciprocal repression, of Otx2 and Gbx2. En1 is expressed in both the mid- and hindbrain regions. b. The genetic interactions among Otx2, Gbx2, Fgf8, Wnt1 and En1. En1, engrailed 1; Fgf8, fibroblast growth factor 8; Gbx2, gastrulation brain homeobox 2; Otx2, orthodenticle homologue 2; Wnt1, wingless-related MMTV integration site 1.

Otx2 homologous-recombinant null mice show some defects in gastrulation and fail to develop forebrain, midbrain and anterior hindbrain. In complete absence of Otx2 no anterior head structures form and the entire brain rostral to rhombomere3 is missing. On the other hand ablation in Gbx2 gene leads to rostralization of the hindbrain. The anterior hindbrain corresponding to rhombomeres 1-3 does not form and the midbrain expands up to the rhombomere 3 / 4 boundary in these mutants.

Misexpression of Otx2 and Gbx2 will interfere with proper positioning of MHO (Suda, et al., 1997). In mice which have only one functional allele of Otx (Otx1^{-/-};Otx2^{+/-}) and in mice that express aberrant Gbx2 in the caudal midbrain, a rostral shift of the mid/hindbrain genes FGF8, Gbx2, Wnt1 and En1/2 is accompanied by a caudal repression of Otx2 at early somite stages (Acampora, et al., 1997; Millet, et al.,

1999). The mice consequently show at least a transient transformation of the midbrain and caudal forebrain to rostral hindbrain fates. On the other hand, the *Gbx*^{-/-} mice and mice that ectopically express *Otx2* in the rostral hindbrain, display a caudal shift of *Otx2*, *Wnt1* and *FGF8* expression accompanied by a rostral repression of *Gbx2* (Broccoli, et al., 1999). These result in at least a partial transformation of rostral hindbrain to mesencephalic fate.

Overall, a minimal dosage of *Otx2* is required for the proper regionalization of the anterior neuroectoderm in both forebrain and midbrain territories (Martinez-Barbera, et al., 2001). On the other hand, *Gbx2* plays permissable role for development of the anterior hindbrain (Li, 2001). (Figure 5)

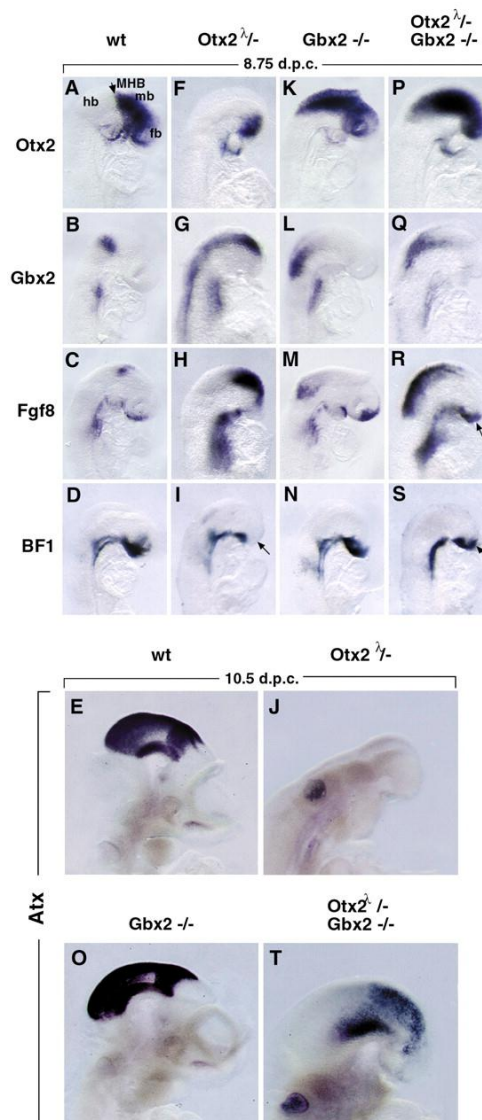


Figure 3. minimal amount of Otx2 protein suffices to displace Fgf8 and Gbx2 expression posteriorly and to induce forebrain- and midbrain-specific gene expression. (A-T) Whole-mount in situ hybridisation of wild-type (A-E), *Otx2*^{Δ/-} (F-J), *Gbx2*^{-/-} (K-O) and *Otx2*^{Δ/-}; *Gbx2*^{-/-} (P-T) embryos with *Otx2* (A,F,K,P), *Gbx2* (B,G,L,Q), *Fgf8* (C,H,M,R), *Bf1* (D,I,N,S) and *Atx* (E,J,O,T) probes shows that *Otx2*^{Δ/-}; *Gbx2*^{-/-} double mutants recover a wide-spread expression of *Otx2* (P), a posterior displacement of *Gbx2* (Q) and *Fgf8* (R) transcripts and the neuroectodermal expression of both the forebrain gene *Bf1* (compare arrow in I and S) and the midbrain gene *Atx* (T). The arrow in R indicates *Fgf8* expression in a region corresponding to the presumptive ANR

The role of Sonic Hedgehog in induction of mesDA neurons

Sonic hedgehog (*Shh*) gene belongs to hedgehog family. It seems to start signaling on 7.5 – 9.5 and express on E8.5 – 11.5 (Perez-Balaguer, et al., 2009). Organization of neuronal population (dopaminergic neurons and the oculomotor complex) and differentiation of Midbrain basal plate neural, are among the function of *Shh*. *Shh* also acts as a growth factor promoting cell proliferation and survival in midbrain and caudal forebrain. The regional specification and differentiation of the ventral spinal cord depends on *Shh* signaling. SHH signaling is transcriptionally mediated by the Zinc-finger proteins of Gli family (Jacob and Briscoe, 2003). The Gli proteins are regulated in the cytoplasm via multiple distinct molecular mechanisms (Cayuso and Marti, 2005), including the PKA (cyclic AMP dependent protein kinase) as a negative regulator. PKA-mediated phosphorylation would make Gli repressor forms (Cayuso, et al., 2006). So Gli proteins can go to nucleus and repress or activate transcription of specific target gene (Cayuso and Marti, 2005). (Figure 6)

Shh induces the differentiation of floor plate motorneurons and ventral interneurons (Ericson, et al., 1996). Elimination of *Shh* function prevented the differentiation of floor plate, motorneurons and most classes of ventral interneurons (Chiang, et al., 1996; Marti, et al., 1995). Progressive changes in SHH concentration generate five

molecularly distinct classes of ventral progenitor cells at defined positions within the ventral ventricular zone (Cayuso and Marti, 2005).

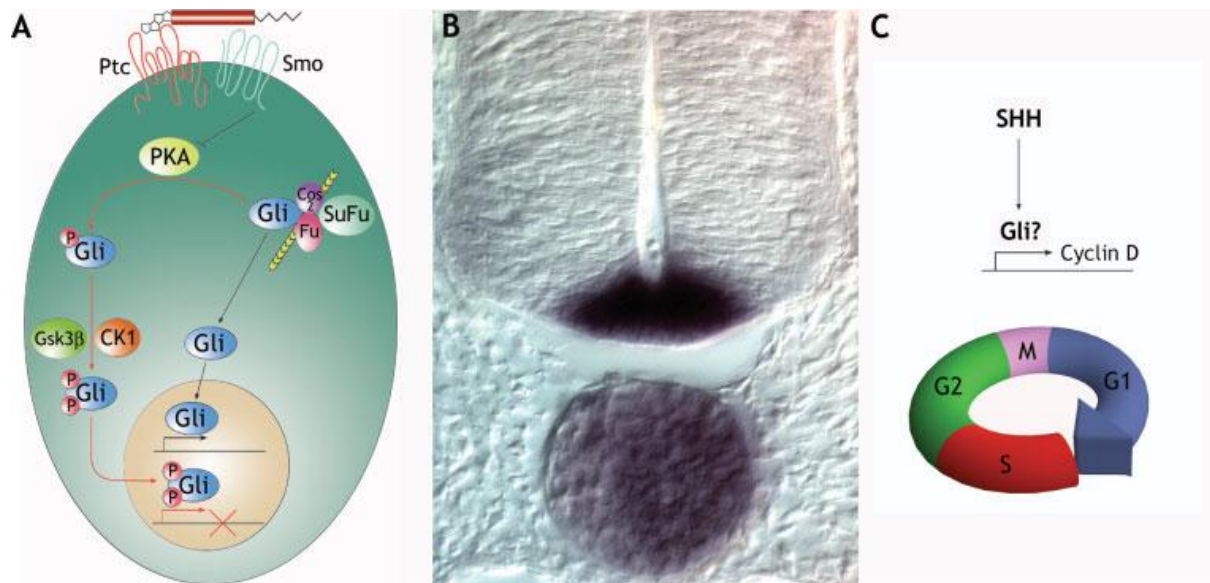


Figure 6. Shh pathway role in the proliferating neural precursors.

(A) Schematic representation of the principal components of the Shh pathway. (B) In situ hybridization of a spinal cord section showing expression of Shh in the notochord and in the floor plate. (C) Transcriptional targets of the Shh–Gli signaling (cyclin D) that can promote G1/S cell cycle progression.

The graded Shh signaling induces the expression, in the basal plate, of class II homeodomain factors and the repression of class I homeodomain factors. The Nkx family of transcription factors represents the class II homeodomain factors. The mesencephalic territory, as in the case of the spinal cord and hindbrain needs the correct positioning of the Nkx family of transcription factors along with the basal plate to generate the appropriate progenitor domains for the differentiation of its distinctive neural population. In addition, Shh is responsible for the induction and maintenance of ventral neural tube and also appears to be required for the normal growth of the diencephalic and mesencephalic primordial by sustaining proliferation and survival of both ventral and dorsal precursors. Not all neuronal populations are depends on Shh for their maintenance, because it has been shown that while in absence of Shh aculomotor complex is lost and the dopaminergic population is affected, the red nucleus is maintained (Perez-Balaguer, et al., 2009). Shh and FGF8 have essential

role in the induction of mdDA neurons. Shh and FGF8 establish a conjunction with TGF β and induce TH-positive cells in culture {Rousseau, 2004 #2366}.

There is a specific genetic pathway for the specification of the mesencephalic red nucleus and it is reported that Foxa2 maybe is the gene that controls the Shh signaling pathway (Mavromatakis, et al.).

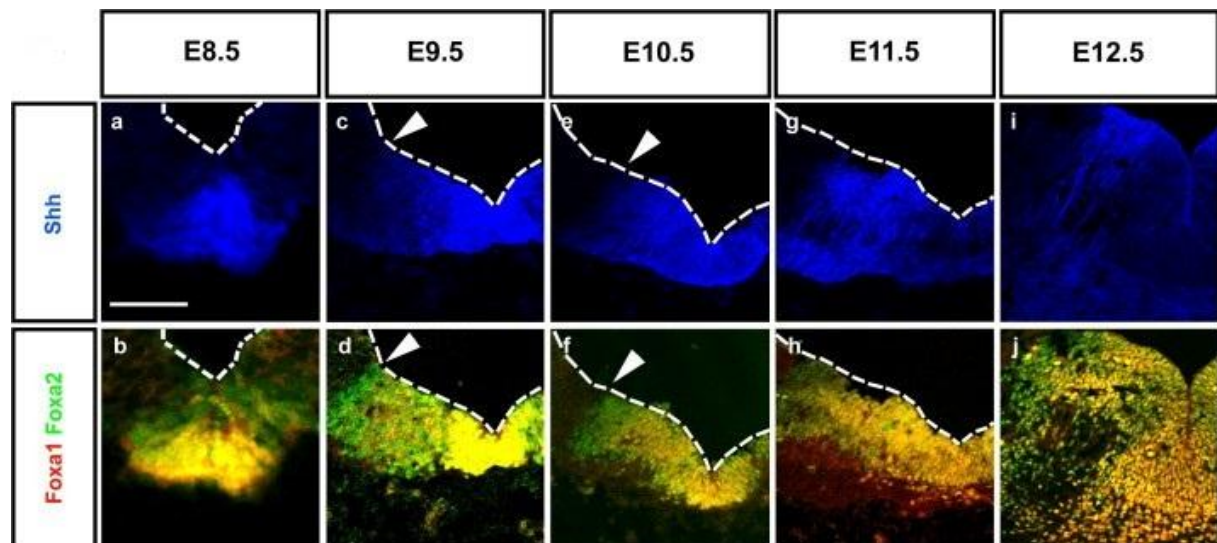


Figure 7. Coexpression of Foxa1, Foxa2 and Shh genes and their similar patterning in the floor plate of the midbrain in mouse embryo;

Foxa1, Foxa2 and Shh are expressed in a similar pattern in the floor plate of the midbrain of mouse embryos at E8.5 (a and b). The expression three proteins in the midbrain overlap to a large extent at E9.5 and E10.5 (c and f), but for a group of cells near the alar/basal boundary (d and f) only Foxa2 has been expresses. At E11.5 and E12.5, although the expression of Foxa1 and Foxa2 remains unchanged but, Shh expression in the floor plate subsequently decreases (g and j).

So seeing overlap in expression of Foxa2, Foxa1 and Shh, suggests that may be there is a cross-regulatory interactions between these genes during the embryonic stages (Mavromatakis, et al.). (Figure 7)

In the Shh knockout mice, the floor and basal plate are completely absent (Perez-Balaguer, et al., 2009). Several factors are involved in the Shh signaling pathway.

For example absence of *smoothened* (smo), which is one of receptors of shh, after 8 days of gestation resulted in the absence of the oculomotor complex and dopaminergic populations (Blaess, et al., 2006).

As discussed, Shh is necessary for cell proliferation, differentiation and neuroprotection in the central nervous system. In a study on the 6-OHDA model of PD, rat PD model, stereotactic injection of N-terminal products of SHH into the striatum 24 h after a striatal 6-OHDA lesion resulted in attenuation of the behavioral deficits, reduced the loss of dopamine neurons in the substantia nigra and the loss of dopamine fibers in the striatum.

Key role of FGF8

FGF8 belongs to fibroblast growth factor family. The most important role of FGF8 is the early patterning of the vertebrate's midbrain and cerebellum. In addition the duration of FGF8 isthmic organizer expression is a key to make difference in patterning of tectal-isthmo-cerebellum structure. Patterning of midbrain is all about importance of substantia nigra pars compata and other part.

FGF8 is in interaction with Shh, this interaction would induce the precursor cells of mesDA neurons in the ventral midbrain. In 2004 it was shown that FGF8 is participating in a complex regulatory network. Which includes the regulation of its own receptors and inhibitors. In addition to the role of FGF8 in the isthmic organizer expression is a key to make difference in patterning of tectal-isthmo-cerebellum structure. From FGF gene family, FGF8a, FGF8b, FGF18 have important roles in embryonic zone (Sato, et al., 2004). A strong FGF signal mediated by FGF8b activates the Ras-ERK pathway and this is sufficient to induce cerebellar development. FGF8b activates ERK strongly in all region mes/r1 and di. But misexpression of FGF8a would activate ERK in di only (Sato, et al., 2004)(figure 8). Proliferation and differentiation is two of molecular path ways. In these path ways cellular responses should be induced by signals to start their job. Signaling through a

receptor tyrosine kinase(RTK) is transduced by the Ras-extracellular responses in these kind of molecular **path ways** (Katz and McCormick, 1997). Phosphorylation of threonine and tyrosine residues which lie adjacent to each other in the unique TEY sequence, would activate the ERK (Corson, et al., 2003).

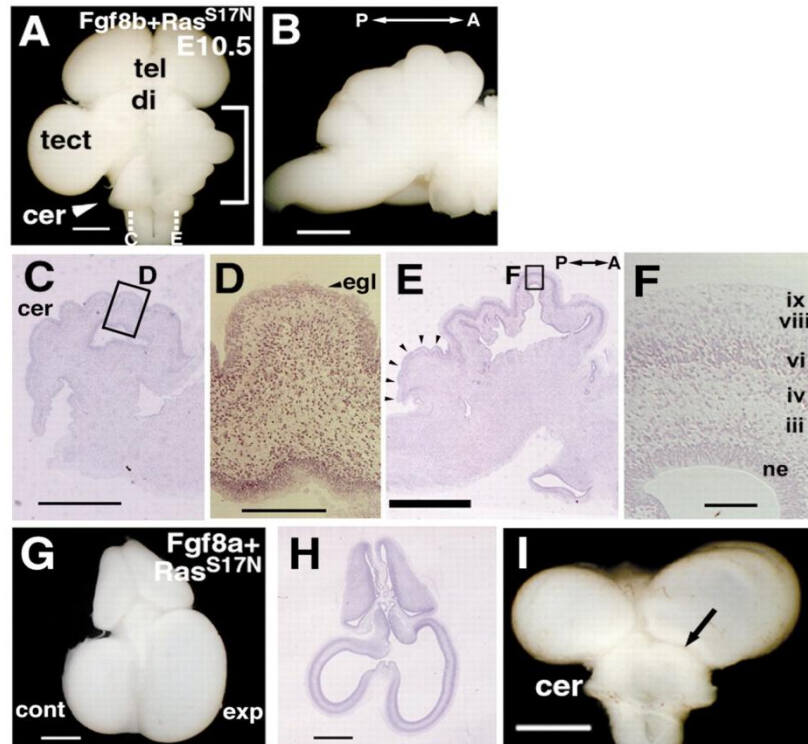


Figure 8. Co-transfection of Fgf8b/a with RasS17N.

(A-F) Dorsal view (A) and lateral view (B), parasagittal section at the line indicated on (A) as (C) and (E) (C,E) of E10.5 brain co-electroporated with Fgf8b and RasS17N at the 10-somite stage. (D,F) higher magnification of (C,E, respectively). The swelling in the mesencephalic region and anterior metencephalic region of the experimental side have sulci on the surface, and look like cerebellum (A,B). Histologically, however, the swelling in the mesencephalon and the anterior metencephalon of the experimental side do not have an external granular layer, which is seen in the cerebellum of the control side (C,D), and has tectal architecture (E,F). The posterior part of the metencephalic region consists of cerebellar structure (arrowheads on E). Antero-posterior direction is indicated by the double-headed arrow on (B,E). Dorsal view (G,I) and horizontal section (H) of the brain co-electroporated with Fgf8a and RasS17N. The tectum has extended to the diencephalic territory (G,H). Tectum differentiated ectopically in the metencephalon (I, arrow). tel, telencephalon; di,

diencephalon; tect, tectum; cer, cerebellum; egl, external granular layer; tect, tectum; ne, neuroepithelial layer; roman numbers on (F) indicate the tectal layers. Scale bars: 4 mm (A,B,C,E,G,H,I); 200 μ m (D,F)(Sato and Nakamura, 2004).

One of important roles of FGF8 is the way that it regulates itself! First FGF8b would induce the expression of FGF18 then two intracellular inhibitors of receptor tyrosine kinase-signaling are expressed in the board domain across the MHB which is include the FGF receptor Sprouty1 and 2. Then Spry 1 and 2 are directly induced by FGF8b (Prakash and Wurst, 2004).

FGF8, FGF17 and FGF18 are products of mid/hind brain organizer and they are important for early patterning of the vertebrate midbrain and cerebellum. FGF8 plays a key role in mes/r1 development by being upstream of mes/r1 determination genes. It has been shown that mes and metencephalon development would be regulating by molecules produced by a signaling center (IsO) (Sato, et al., 2004). FGF8 and WNT1 are the key component of IsO signaling activity. There are FGF8a and FGF8b. FGF8b can transform the midbrain in to cerebellum fate and FGF8a can promote midbrain development (Panhuysen, et al., 2004; Sato, et al., 2004).

In addition FGF17/18 and FGF2/3 are important in that development. Development of r1 tissue needs a powerful FGF8/17/18 signaling (Prakash and Wurst, 2004). And the strength of this signaling determines which one should develop, r1 or mes While the FGF signaling is powerful and r1 development is in progress, Ras-ERK pathway would be activated, but when the signaling is not powerful, Ras-ERK pathway would not get active and Otx2 would express and tectum development occurs (Sato, et al., 2004).

FGF8a and FGF8b have different activities and functional roles. FGF8a misexpressed transgenic mice in the mesencephalon (mes) show overgrowth of the diencephalic/mesencephalic region. FGF8b misexpressed transgenic mice shows up-regulation of rhombomere1(r1)/isthmus markers (Gbx2 and Fgf8) and repression of Otx2 in diencephalic/mesencephalic regions (Sato, et al., 2004).

Deletion of *fgf8* results in absence of the entire midbrain and anterior hindbrain in both dorsal and ventral domains (Prakash and Wurst, 2004). The ectopic expression of FGF8a in the mouse midbrain and caudal forebrain using a WNT1 regulatory element rescued this effect (Panhuysen, et al., 2004).

En and PAX genes are necessary for mid/hindbrain development

These two genes are expressed in early stages of somitogenesis and have a prominent role in development of midbrain and rostral hindbrain region of the neural plate.

En1 belongs to the engrailed homeobox gene family and its expression in the midbrain starts on the embryonic day 8 (E8). The Engrailed gene was originally described in the fruitfly as a selector gene. Mutation in this gene would cause a homeotic transformation of the posterior wing compartment of the fruitfly into an anterior compartment (Prakash and Wurst, 2004) (Wurst and Bally-Cuif, 2001). These genes have been widely studied in a variety of species including annelids (Wedeen and Weisblat, 1991), mollusks (Wanninger and Haszprunar, 2001), insects, crustaceans (Duman-Scheel and Patel, 1999; Patel, et al., 1989), echinoderms (Lowe and Wray, 1997), chordates such as amphioxus (Holland, et al., 1997), and multiple vertebrates species (Ekker, et al., 1992; Koster, et al., 1996) including humans (Alavian, et al., 2008; Barak, et al., 2003; Zec, et al., 1997).

Their involvement in patterning of the early mouse embryo was shown based on the analysis of the mutant mice (Simon, et al., 2004). Both engrailed homologs in vertebrates (En1 and En2) are expressed during early neural development across the MHB in the caudal midbrain and rostral hindbrain, also in a region where mesDA neurons arise (Prakash and Wurst, 2006). Furthermore, both En1 and En2 continue to be expressed within the SN and VTA of the postnatal and adult mouse brain

(Davis and Joyner, 1988) (Simon, et al., 2004). En genes perform an essential function during the development. First when the basic body plan is laid down, En genes would regionalize the embryo. Then they have a role in neuronal specification (Simon, et al., 2001) (Lundell, et al., 1996) (Harzsch, et al., 1998).

Also it was hypothesized that En1 and En2 may have an important role in induction, differentiation and maintenance of mesDA neurons (Prakash and Wurst, 2006). To test the last hypothesis, both single and double mutant En1^{-/-} and En2^{-/-} mice were analyzed for expression of Th. It has been shown that in single En1^{-/-} mutant or single En2^{-/-} mutant mice, mesDA neurons appeared in the SN and VTA. Furthermore, in double mutant mice for both genes mesDA neurons are completely absent in SN and VTA (Simon, et al., 2001). It has been shown that the presence of mesDA neurons in compound heterozygote/homozygote mice is depend on En1 expression and it is depend on the gene dosage too. Thus, the SN and VTA were substantially reduced in En1^{-/-}; En2^{+/-} mice but appeared normal in En1^{+/-};En2^{-/-} mice (Prakash and Wurst, 2006). The role of En1 is more prominent in the development of the midbrain. While the En1 ^{-/-} mice display deletion of the dorsal and the ventral parts of the midbrain and rostral hindbrain (Wurst, et al., 1994), the phenotype of the En2^{-/-} mice is limited to minor cerebellar defects (Millen, et al., 1994). Deletion of all four alleles of engrailed (En1^{-/-}; En2^{-/-}) results in a complete deletion of the midbrain/hindbrain region and loss of early expression of Wnt1, Fgf8 and Pax5 (Sato and Nakamura, 2004). The engrailed genes perform an important role in neural specification and in maintenance of functional midbrain hindbrain organizer through regulation of Fgf8 expression (Sato and Nakamura, 2004).

Other functions of the engrailed genes include regulation of the expression of α -synuclein, which is genetically linked to Parkinson's disease and repressing Pax-6 as a forebrain specific gene. α -synuclein is the first known genetic link to relative of PD (Polymeropoulos, et al., 1996; Polymeropoulos, et al., 1997). This gene is important in the normal synaptic membrane-associated processes. It has been shown that it is

an activity-dependent negative regulator of dopaminergic neurotransmission (Abeliovich, et al., 2000). While the loss of function mutations can cause PD, the association of triplication of its locus to familial PD suggests that the survival of nigral DA neurons depends on the right level of its expression (Singleton, et al., 2003).

In En-1 null mice the expression of α -synuclein by midbrain dopaminergic neurons is diminished. Also in engrailed double mutants the expression is absent. although the TH-positive neurons are still present. These explain that expression of α -synuclein in ventral midbrain dopaminergic neurons would be regulate by En-1 and En-2 (Simon, et al., 2001).

Pax genes, are also the regulatory targets of engrailed. They belong to the paired box family (tissue specific transcription factor) which is involved in induction of fgf8 transcription and cooperate in establishment and maintenance of the MHO (Prakash and Wurst, 2004). Pax genes are expressed on presomitic stages before all other MHO genes (Ye, et al., 2001). Pax2^{-/-} embryos do not properly develop the mid-/hindbrain region (Bouchard, et al., 2000). The Pax5^{-/-} embryos show a partial deletion of the inferior colliculli and slightly enlarged cerebellum. In Pax2^{-/-} ; Pax5^{-/-} embryos, most of the midbrain and cerebellum is missing (Schwarz, et al., 1997).

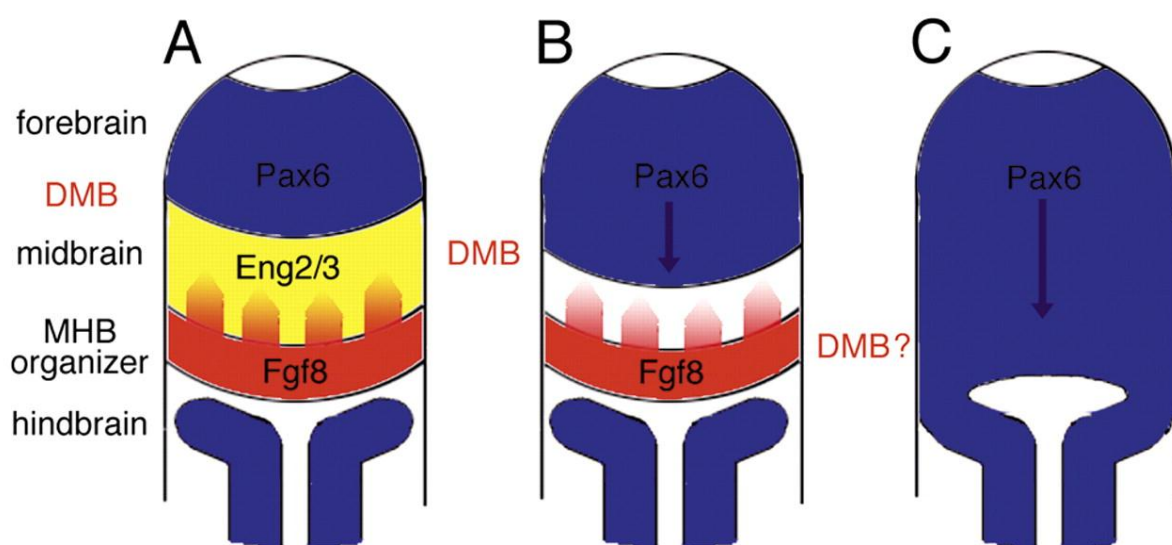


Figure 9. Function of eng2/eng3 and fgf8 in anterior/posterior patterning of the anterior neural plate.

Pax6.1 (blue) demarcates the forebrain and hindbrain, whereas expression of eng2/eng3 (yellow) and fgf8 (red) can be observed in the midbrain/MHB organizer territory (A). Eng2/Eng3 displays the cell-autonomous signal maintaining midbrain fate, whereas Fgf8 acts non-autonomously in the midbrain territory visualized by red arrows. (B) Loss of Eng2/Eng3 causes a posterior shift of the diencephalic-mesencephalic boundary (DMB) (blue arrow). Fgf8 expression is unaffected at least until 10 ss, suggesting a partial repression of the forebrain expansion. Loss of both signals Eng2/Eng3 and Fgf8, causes a loss of midbrain identity and a fusion of forebrain and hindbrain domain (C). Only a dorsal patch of cells remains free of pax6.1 expression. (Scholpp, et al., 2003)

Foxa1 and Foxa2 Are Required for the Maintenance of Dopaminergic Properties in Ventral Midbrain Neurons at Late Embryonic Stages

Foxa1 is a member of the forkhead class of DNA-binding proteins. Albumin and transthyretin are examples of transcriptional activators for liver-specific transcripts, which these hepatocyte nuclear factors are responsible for. In addition, these proteins have a pioneer factor role in interaction with chromatin. Pioneer factors are those transcription factors which can bind to dense chromatin.

Forkhead box protein A2 (Foxa2) which is also known as the hepatocyte nuclear factor 3-beta (HNF-3B) and transcription factor 3B (TCF-3B), is encoded by the Foxa2 gene (Lee, et al.; Wang, et al., 2002). Foxa2 has a similar role to that of Foxa1. It activates albumin and transthyretin which, as mentioned, are liver-specific genes and it also has interaction with chromatin. It is interesting to note that Foxa2 is linked to diabetes of the young. Experiments show that there are various isoforms which transcribe this gene ([Entrez Gene: forkhead box A2](#)).

Foxa1 and Foxa2 are expressed throughout the life of mesDA neurons (Thuret, et al., 2004). They have an essential role in specification and early development of mesDA neurons (Ferri, et al., 2007). The Foxa genes are expressed in adult mesDA neurons but their precise role is not clear yet (Stott, et al.).

In VM, between E14.5 and 18.5, Foxa1 and Foxa2 were expressed (figure10. A,B, respectively). Both Foxa1 and Foxa2 genes expressed in more nuclei than just mesDA neurons (figure10.A, B). After birth both genes were expressed by mesDA neurons at all time-points analyzed through to P60 (figure10.C-E) Meanwhile Foxa1 expressed specifically in the adult VM, Foxa2 was maintained in additional VM nuclei (figure10.C-E). Both genes were present in all of mesDA neurons analyzed at all time points which have been examined. Interestingly, Foxa1 and Foxa2 expressed specifically in nuclei and they had not seen in cytoplasm(figure10.B,C, inset). In conclude, persistent expression of these two genes during the maturation of mesDA system shows this result; Foxa1 and Foxa2 have essential role in differentiation and/or maintenance of mesDA neurons (Kittappa, et al., 2007; Stott, et al.).

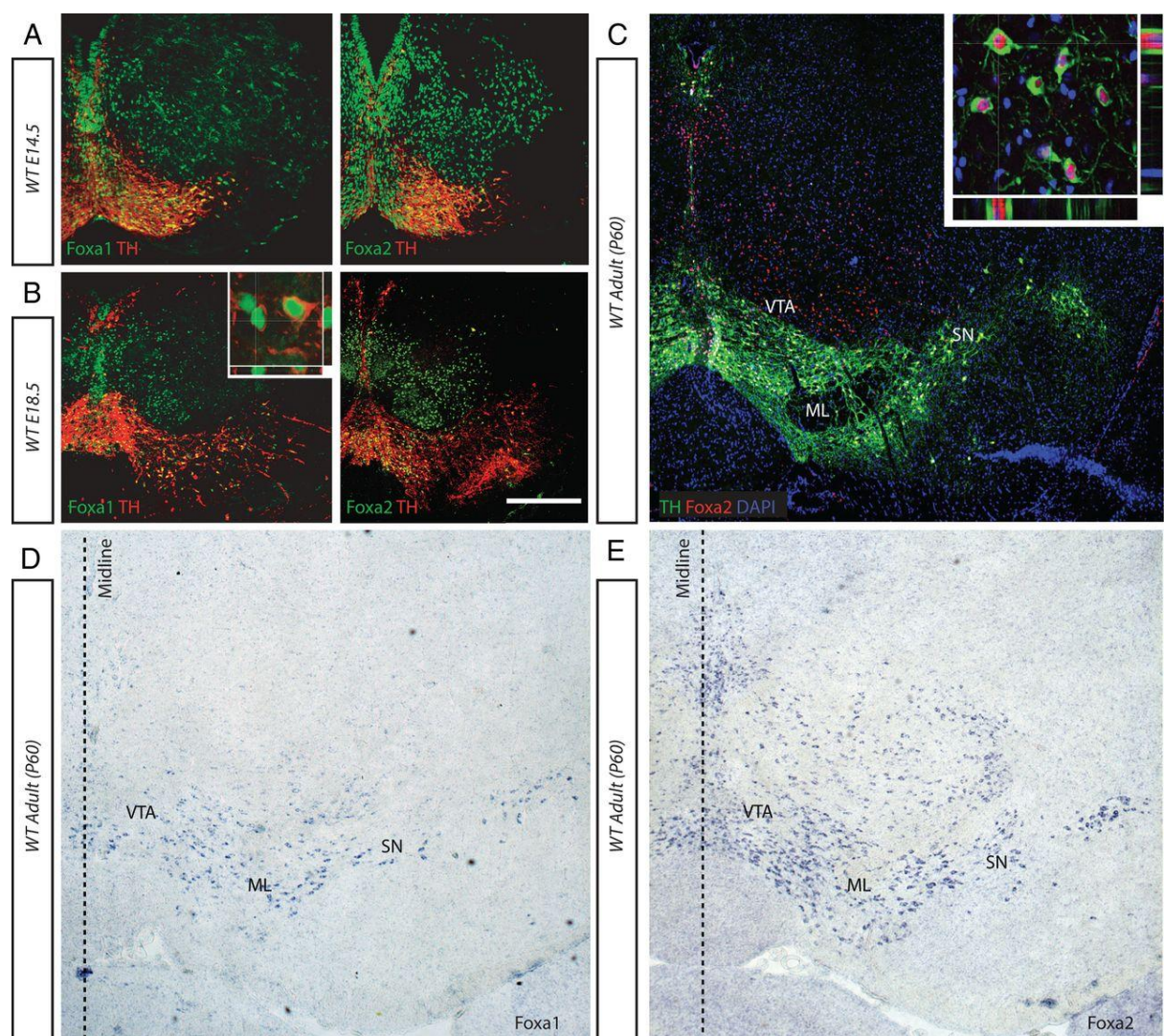


Figure 10. The expression of the Foxa genes in the mesDA neurons is maintained into adulthood.

A, B, Immunological staining with Foxa1 and Foxa2 antibodies demonstrates their expression patterns on coronal sections of VM at E14.5 and E18.5 (*A, B*, respectively). *C, E*, Immunological and *in situ* stainings demonstrate that both Foxa2 are expressed in the ventral midbrain of 60-d-old adult mice (*C, E*, respectively). *D*, Foxa1 expression was detected in the mesDA region only by *in situ* hybridization, as the Foxa1-specific antibody did not work in adult sections. Foxa1 and Foxa2 expression was localized to the nucleus both embryonically (*B*, inset) and postnatally (*C*, inset), respectively. ML, Medial lemniscus. Scale bar: (in *B*) *A*, 80 μ m; *B*, 100 μ m; *C, D*, 50 μ m (Stott, et al.).

The mesDA neurons would lose of their dopaminergic phenotype in lack of Foxa1 and Foxa2 expression. It has been shown in examined mice that the cells remain in the mesDA domain of VM and still present in aged mice (≥ 18 months old). But those mice revealed that they are not able to express most of the mesDA markers. By way of conclusion, both Foxa genes are critical for the maintenance of the mesDA phenotype (Stott, et al.).

WNT1 acts as a regulator of proliferation of specific precursor population in developing mid/hindbrain

Wingless-type MMTV integration site family member 1 (Wnt1) belongs is expressed in caudal midbrain at E8 and E9.5. The Wnt1 signaling pathways involve binding of Wnt proteins ligands to a Frizzled family receptors (Nusse and Varmus). The two major Wnt1 signaling pathways are the canonical and the noncanonical pathways. In canonical pathway beta-catenin has an important role but in noncanonical pathways beta-catenin is not involve (Figure 11).

Figure 11. Three Wnt1 signaling pathways

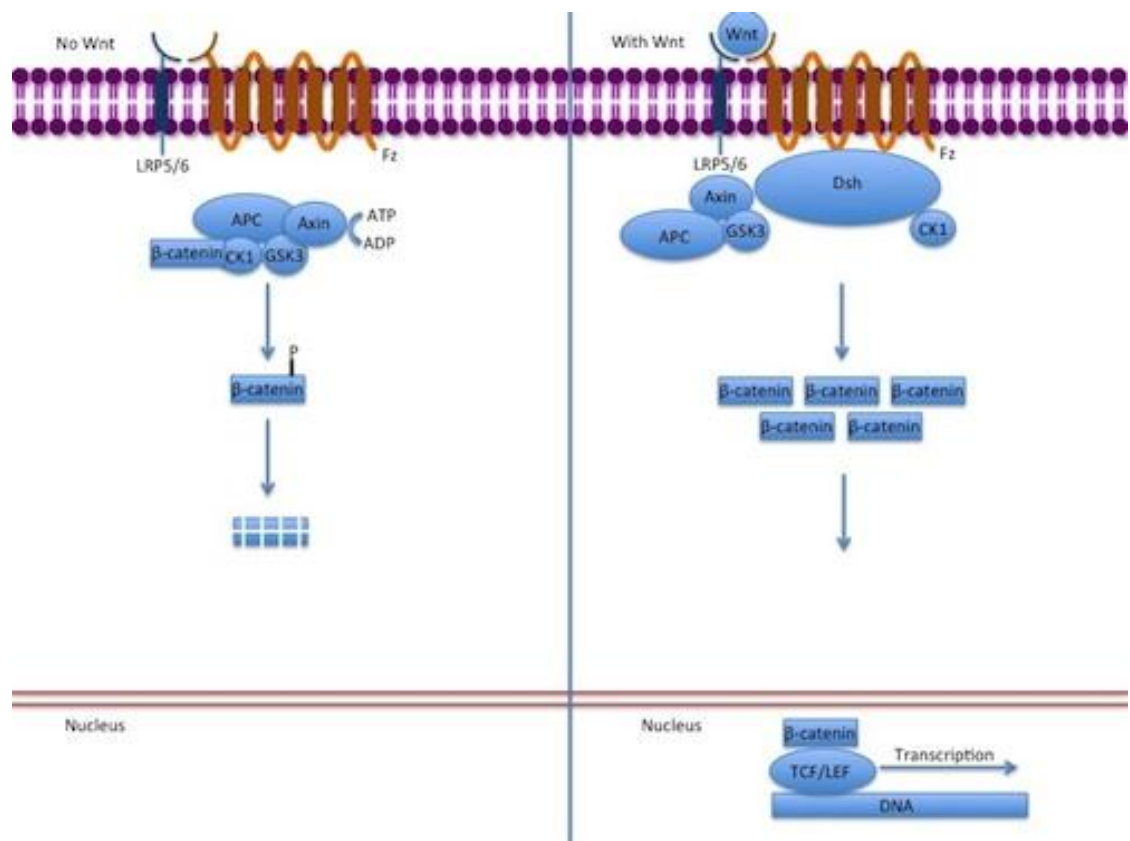


Figure11a. Canonical pathway

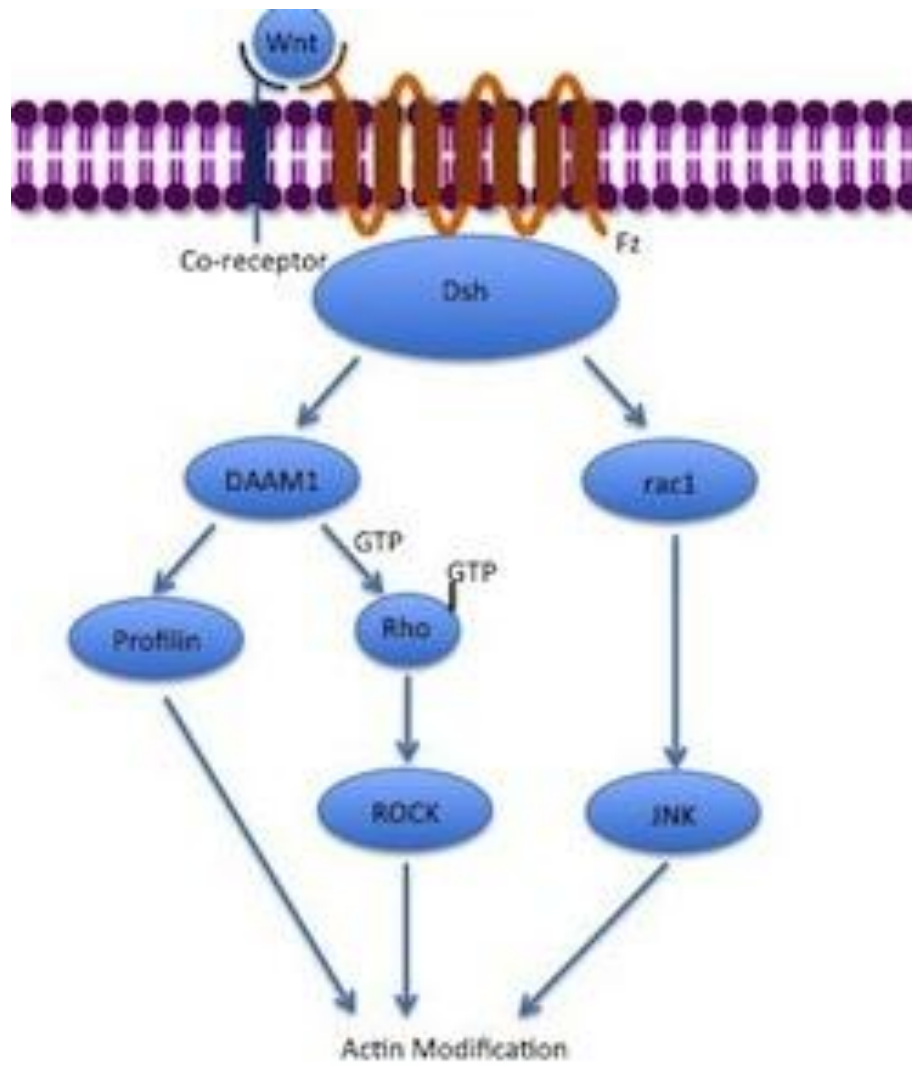


Figure11b. noncanonical planar cell polarity pathway

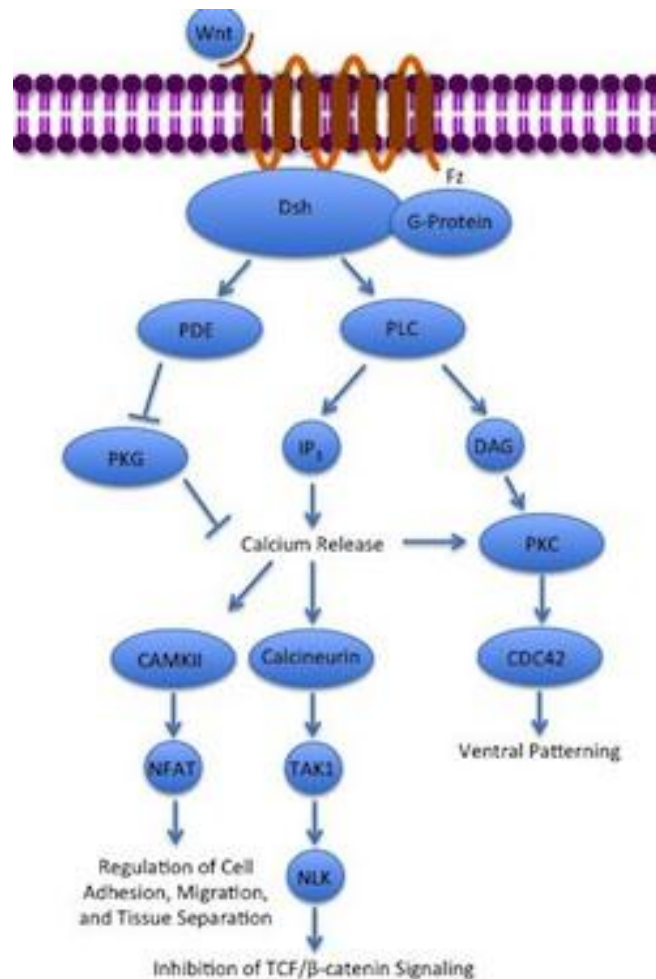


Figure11c. Noncanonical Wnt/calcium pathway

Wnt1 is essential for proper development of the entire mid-/hindbrain region. Wnt1 has several modes of action. In one study Wnt1 was expressed ectopically under the control of En1 promotor. This strategy resulted in an ectopic overexpression of Wnt1 in the midbrain and the ectopic expression in the rostral hindbrain from E8.5 until adulthood. This transgenic model has the advantage of ectopically expressing Wnt1 in a suitable spatiotemporal manner for studying its function specifically during mid-/hindbrain development. The dorsocaudal midbrain of the transgenic animal was dramatically enlarged in size (Panhuysen, et al., 2004). Additionally, Wnt1 affects on cellular and neuronal sizes and has a several other tissue-specific functions such as

regulation of cell proliferation, control of tissue patterning, Cell fate determination, Cell migration, Cell polarity and apoptosis (Panhuisen, et al., 2004).

Wnt1 expression in the mid/hindbrain start with Otx2, Gbx2 and En1 for mid/hindbrain. Inactivation of this gene results in deletion of the entire mid-/hindbrain region and loss of En1 and fgf8 expression and ectopic expression on E8.5 shows dorsocaudal midbrain is dramatically enlarged in size. Misexpression of Wnt1 in chick embryos caused widening of the FGF8 domain non cell autonomously (Matsunaga, et al., 2002).

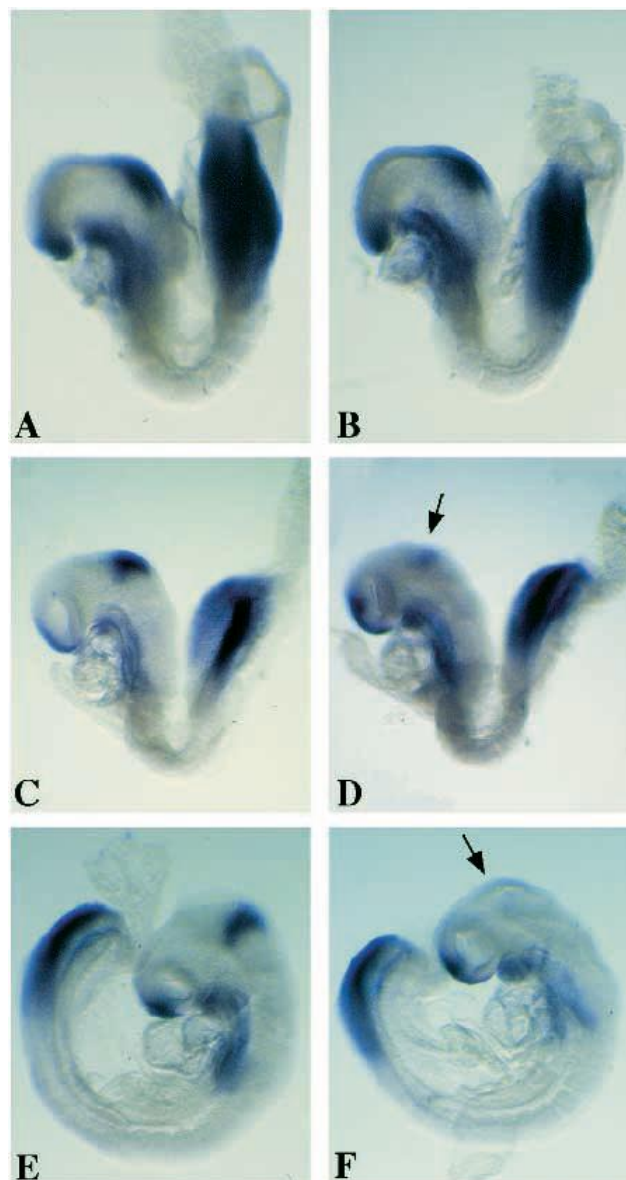


Figure 4. Fgf8 expression in Wnt-1 mutant embryos.

(A,B) Fgf8 expression appears identical in the mes-met region of wild-type (A) and mutant (B) embryos at the 6-somite stage, indicating that Wnt-1 is not required for activation of Fgf8 expression. (C,D) Comparison of wild-type (C) and mutant (D) 9-somite embryos reveals that by this time mesencephalon development is reduced in Wnt-1 mutants. Fgf8 expression in the metencephalon (arrow) is also markedly reduced. (E) By 15 somites Fgf8 expression in wild-type embryos is restricted to a narrow band of cells immediately posterior to the isthmus. (F) In a 14-somite mutant embryo the metencephalon is still present (arrow) but metencephalic Fgf8 expression is completely abolished (Lee, 1997).

Wnt5a; promotion or inhibition of DA progenitor

Wnt5a is another member of the Wnt family. It is a morphogen that activates the Wnt/planar cell polarity (PCP) pathway. Wnt5a serves several functions during development (Andersson, et al., 2008).

Wnt1 and Wnt5a, activate different groups of Wnt signaling and individually regulate distinct perspectives of development of midbrain dopaminergic (DA) neuron. However, several of their functions and interactions have been cleared. It has been shown that loss of *Wnt1* results in loss of *Lmx1a* and *Ngn2* expression, and also agenesis of DA neurons in the midbrain floor plate. In *Wnt1*^{-/-} mutant mice a few ectopic DA neurons are remarkable because they emerge in the basal plate of mice. In those mice *Lmx1a* is ectopically expressed. These results show that *Wnt1* consonants DA specification and neurogenesis in vivo (Andersson, et al.).

Analysis of *Wnt1*^{-/-};*Wnt5a*^{-/-} mice have showed that a greater loss of Nurr1⁺ cells and DA neurons than in single mutants, revealing that both *Wnt1* and *Wnt5a* interact genetically. Collaboration for promoting midbrain DA neuron development in vivo is another result. DA neurogenesis enhance by cooperation of Wnt1 and Wnt5a. This cooperation cause developing an application for Wnts family to improve the

generation of midbrain DA neurons from neural and embryonic stem cell (Castelo-Branco, et al., 2003).

In the other and *Wnt5a*^{-/-} mice also displayed a disorder in midbrain morphogenesis. This defect included impairment in midbrain elongation and a rounded ventricular cavity (Andersson, et al., 2008).

Coordinated Wnt actions increase DA neuron development in vivo and in stem cells. Interestingly recent researches suggest that coordinated Wnt administration can be important and useful to improve DA differentiation of stem cells and in addition the development of stem cell-based therapies for Parkinson's disease (Andersson, et al.).

In summary there is a genetic interaction between *Wnt1* and *Wnt5a*. This interaction can controls different stages of midbrain DA neuron development in an antagonistic or cooperative way (Figure 13). There is competition between Wnt1 and Wnt5a for promotion or inhibition of DA progenitor proliferation. Wnt1 cooperated with Wnt5a in regulating morphogenesis, and Wnt5a cooperated with Wnt1 to promote DA neurogenesis (Andersson, et al.).

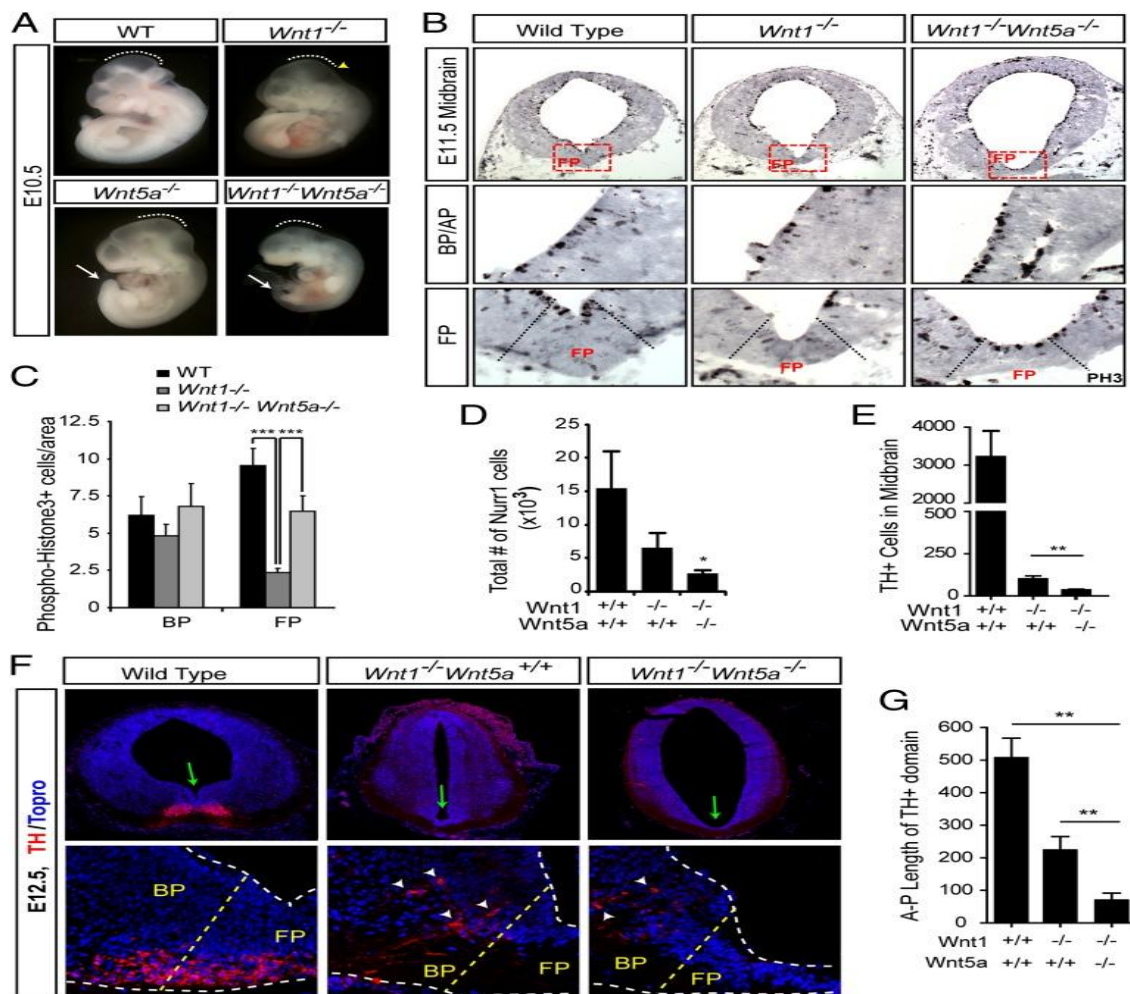


Figure 6. *Wnt1/Wnt5a* double-mutant mice reveal redundant and nonredundant functions during ventral midbrain development.

(A) *Wnt1*^{+/-};*Wnt5a*^{+/-} mice were mated to produce WT, *Wnt1*^{-/-};*Wnt5a*^{+/+} (labeled *Wnt1*^{-/-}), *Wnt1*^{+/+};*Wnt5a*^{-/-} (labeled *Wnt5a*^{-/-}), and *Wnt1*^{-/-};*Wnt5a*^{-/-} mice. At E10.5, *Wnt1*^{-/-} mice display the previously described phenotypes of midbrain deletion and a less distinct isthmus (yellow arrowhead), whereas *Wnt5a*^{-/-} mice display A–P shortening which is most obvious in the tail region (white arrow). *Wnt1*^{-/-};*Wnt5a*^{-/-} double-mutant mice resemble a combination of the two single knockouts, with a shortened A–P axis and a significantly shorter midbrain region (dashed lines). (B and C) Proliferation, assessed by staining for the mitotic marker PH3, is specifically reduced in the FP but not the BP of *Wnt1*^{-/-} mice. Panels show whole midbrain (Top Row) and magnifications of the BP and FP (Middle Row). The areas demarcated by red dotted boxes are magnified in the bottom row. The reduction in the numbers of PH3⁺ cells in the FP of *Wnt1*^{-/-} mice is partially rescued by loss of *Wnt5a*. (D) The total number of Nurr1⁺ cells decreases to a greater extent in double mutants than in single mutants or WT littermates. (E and F) TH⁺ cells are dramatically reduced in number

and are laterally displaced (white arrowheads in *F*) from the FP to the BP in *Wnt1*^{-/-};*Wnt5a*^{+/+} mice. These defects are worsened in *Wnt1*^{-/-};*Wnt5a*^{-/-} mice, in which even fewer cells are found (*E*), and they are positioned further dorsolaterally in the BP (white arrowheads in *F*). The VM hinge point (invagination of the ventricle; green arrow in *F*) is elongated in *Wnt1*^{-/-};*Wnt5a*^{+/+} mice and flattened in *Wnt1*^{-/-};*Wnt5a*^{-/-} mice. (*G*) The decrease of the A–P length of the TH-expressing midbrain domain observed in *Wnt1*^{-/-} mice is exacerbated by loss of *Wnt5a* (Andersson, et al.).

Lmx1a and Lmx1b are required for the specification of dopamine neurons (DNs) during embryogenesis

Lmx1a and Lmx1b are required for the specification of dopamine neurons (DNs) during embryogenesis. Lmx genes belong to LIM homeobox transcription factor family. The LIM homeodomain proteins are co-expressed in DN progenitors. It has been shown that they have a key role in initial steps of DN fate specification and differentiation of DN (Deng, et al.).

Lmx1a, which starts to be expressed at E9, is important for neurogenesis in the medial DN domain and it would convert non-neuronal floor plate cells into neural DN progenitors. Another function of Lmx1a is regulation of Wnt1 expression in midbrain and establishment of Notch signaling that is a highly conserved signaling system which participates in embryogenesis and neurogenesis. In addition Lmx1a involves in differentiation of the DN lineage (Deng, et al.). LMX1A is an early activator in mesDA neuronal differentiation and is crucial for activating Msx1. Both LMX1a and Msx1 transcription factors cooperate to each other for activation of Ngn2. Activation of Ngn2 and blockade of Nkx6.1 activation, allows neuronal generation before these neurons enter the mdDA terminal differentiation pathway. pathway starting with LMX1A through to MSX1 and NGN2 is essential for the normal development of mdDA neurons (Smidt and Burbach, 2007).

On the other hand Lmx1b is expressed on E10.5 and it is involved in expression of Phox2a and inhibition of Sim1 for control of neural fate determination in midbrain. Lmx1b influences the differentiation of multiple neural subtypes in the ventral midbrain and controls the generation and influences the temporal specification of ocular motor neurons (OMNs) and red nucleus neurons (RNNs) in the ventral midbrain (Figure 14). Establishing the isthmus at the mid-/hindbrain boundary is one of early functions of Lmx1b genes.

As mentioned above, one of the early factors involved in mdDA neuronal generation is LMX1B. Early studies on the Lmx1b knockout mice showed that mesDA neurons with a different molecular makeup were born in the mesDA neuronal region of these animals. At stage E12.5 there was expression of TH in neurons that did not co-express PITX3 and an ectopic population was present that expressed PITX3 but not TH. These discoveries suggest that the Lmx1b is essential for the generation and proper differentiation of mesDA neurons. In addition, another role for Lmx1b is the regional specification of the midbrain– hindbrain area, probably through the activation of WNT1(Adams, et al., 2000).

In mutants for these genes lateral DN progenitors would not appear. In Lmx1b mutant; abnormal skeletal development and renal dysplasia as seen in nail patella syndrome. Also it causes the majority of the TH-positive cells in the ventral midbrain. Plus, in mutation of Lmx genes some abnormalities would happen in Wnt signaling. Wnt signaling is discussed in Wnt part of this review (Deng, et al.).

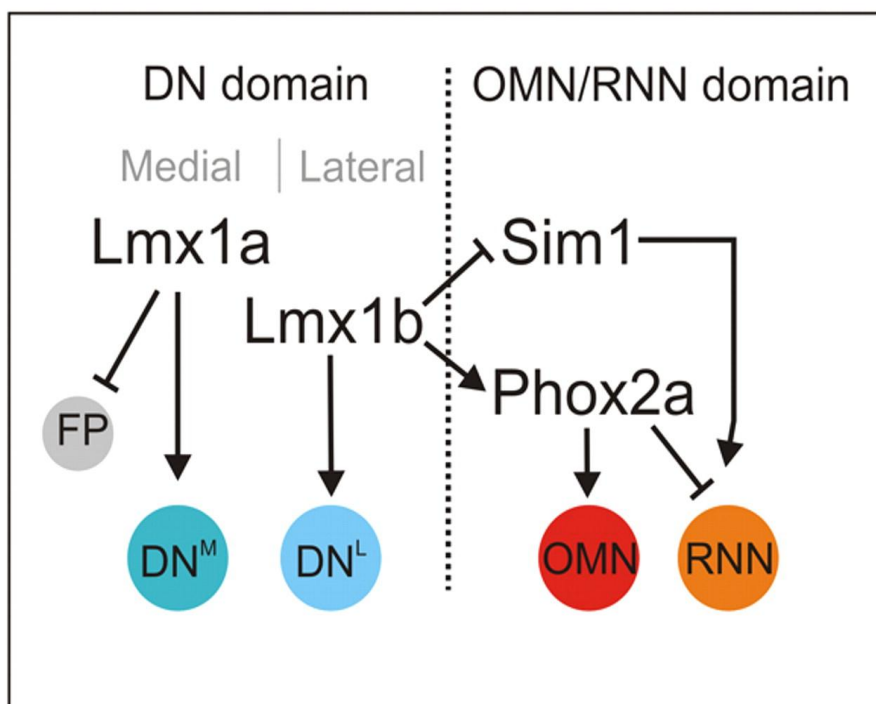


Figure 6. Model outlining specific roles for Lmx1a, Lmx1b and Phox2a in the spatiotemporal control of neuronal fate determination in the midbrain.

Lmx1a and Lmx1b are co-expressed in DN progenitors in the ventral midbrain, and mediate both specific and redundant activities in DN specification. The DN progenitor domain can be subdivided into medial (DN^M) and lateral (DN^L) subpopulations of cells. There is specific requirement for Lmx1a in regulating the onset of neurogenesis in medial cells, and this process involves establishment of Notch signaling and a concomitant conversion of non-neuronal floor-plate (FP) into neuronal DN progenitors. Lmx1b has little effect on midline neurogenesis but is instead required for the establishment of lateral DN progenitors, and for suppressing Sim1 expression and RNN fate within the DN progenitor domain. This spatial patterning activity of Lmx1b is probably essential at early time points when Lmx1b is broadly expressed in the ventral midbrain but Lmx1a expression is restricted to floor-plate cells. In addition to its role in the DN differentiation lineage, Lmx1b also controls the sequential generation of OMNs and RNNs by ensuring the activation of Phox2a in progenitors located immediately lateral to DN progenitors. Phox2a and Sim1 are co-expressed within this progenitor pool, and Phox2a is required to promote OMN fate and suppress the generation of RNNs at early developmental stages. Sim1 is likely to contribute to the specification of RNNs within this progenitor pool, but such activity must be subordinated to Phox2a and therefore not revealed until the expression of Phox2a is downregulated (Deng, et al.).

Discussion

The factors mentioned in this work play specific roles during the early development of the midbrain and the mesencephalic dopaminergic neurons. These functions include the regionalization of the midbrain, development of the midbrain-hindbrain organizer, defining the regional identity of the mesDA neurons, induction,

specification and differentiation of dopaminergic neurons. Some of the factors involved in the early development of the midbrain and the mesDA neurons continue to play a role in the postmitotic development and even in the long-term survival and maintenance of the mesDA neurons.

Otx2 and Gbx2 are the two major factors essential for correct positioning of the midbrain. Otx2 defines the prospective forebrain and midbrain and Gbx2 defines the prospective hindbrain region and spinal cord. A minimal dosage of Otx2 is required for the proper regionalization of the anterior neuroectoderm in both forebrain and midbrain territories (Martinez-Barbera, et al., 2001). On the other hand Gbx2 plays a permissible role for development of anterior hindbrain (Li, 2001). Shifting the domains of expression of either gene would result in the improper regionalization of the brain.

During early development, the engrailed genes play a role in maintenance of functional MHO and development of mid-/hind brain boundary. Later on, the expression of the engrailed genes in the midbrain becomes limited to the mesDA neurons as they become specific survival and maintenance factors within the dopaminergic neurons. They play several regulatory roles such as expression of α -synuclein and repressing Pax 6 as a forebrain specific gene.

Foxa1 and Foxa2 are other examples of factors that have an essential role in specification and early development of mesDA neurons (Ferri, et al., 2007) but continue their expression to play a survival function throughout the life of mesDA neurons (Thuret, et al., 2004).

Shh is expressed in the floor plate and is responsible for induction and maintenance of ventral neural tube structure and also appears to be required for the normal growth of the diencephalic and mesencephalic primordial by sustaining proliferation and survival of both ventral and dorsal precursors. The induction of mesDA neurons takes place through the cooperative action of Shh from the floor plate and Fgf8 from

the midbrain-hindbrain organizer. Shh also acts as a growth factor promoting cell proliferation and survival in midbrain and caudal forebrain. But not all neuronal populations depend on Shh for their maintenance, since while the oculomotor complex and dopaminergic populations are affected by the loss of Shh expression, the red nucleus remains intact

The glycoprotein, Wnt1, is important for proper development of the entire mid/hindbrain region. Wnt1 has many tissue specific functions such as regulation of cell proliferation, control of tissue patterning, cell fate determination, cell migration, cell polarity and apoptosis. Wnt1 and Wnt5a, activate different groups of Wnt signaling and individually regulate distinct aspects of development of midbrain dopaminergic (DA) neuron. Wnt1 and Wnt5a have interaction and they can control different stages of midbrain DA neuron development in an antagonistic or cooperative way. There is competition between Wnt1 and Wnt5a for promotion or inhibition of DA progenitor proliferation. Wnt1 cooperates with Wnt5a in regulating morphogenesis, and Wnt5a cooperates with Wnt1 to promote DA neurogenesis (Andersson, et al.).

Lmx1a is the most prominent factor in the intermediate development of mesDA neurons. It is important for neurogenesis in the medial DN domain and it would convert non-neuronal floor plate cells into neural DN progenitors. Lmx1a is responsible for regulation of Wnt1 expression in midbrain and establishment of Notch signaling that is a highly conserved signaling pathway in embryogenesis and neurogenesis. In addition Lmx1a involves in differentiation of the DN lineage. The other member of the Lim homeodomain family, Lmx1b, is involved in expression of Phox2a and inhibition of Sim1 for control of neural fate determination in midbrain. Lmx1b influences the differentiation of multiple neural subtypes in the ventral midbrain and controls the generation and influences the temporal specification of OMNs and RNNs in the ventral midbrain.

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