1. Introduction

1.1 The olfactory system

In the animal world, the detection of environmental odorants by olfaction has been critical for the evolution of successful species. The olfactory system is important not only for food selection, but also for reproductive functions, neuroendocrine regulation, emotional responses, aggression and recognition of predators and prey. An indication of the importance of the olfactory system is shown by the significant proportions (as much as 4%) of the genomes of many higher eukaryotes that are devoted to encoding the proteins that sense smell.

Animal and human olfactory systems constitute phylogenetically very old structures (Martin and Jessell 1995), processing and relaying chemical information from the outside world to higher brain structures. Moreover, from flies to mammals, there is a striking evolutionary convergence towards a conserved organization of signaling pathways in olfactory systems (Hildebrand and Shepherd, 1997). Two olfactory systems have developed in most animals. The common or main olfactory system is the sensor of the environment, the primary sense used by animals to find food, detect predators and prey, and mark territory. A second, or accessory, olfactory system has developed for the specific task of finding a receptive mate. Known as the vomeronasal system, it specializes in recognizing species-specific olfactory signals produced by one sex and perceived by the other, and which contain information about reproductive state and availability.

Odor molecules are transduced by olfactory receptor neurons (ORNs), first order neurons located in the olfactory epithelium within the nasal cavity. The ORNs are bipolar neurons with a single dendrite that reaches up to the surface of the tissue and which send their axons into a region of the forebrain known as the olfactory bulb (OB). Recent molecular-genetic studies using transgenic mice have shown that all the neurons expressing a particular olfactory receptor, no matter where they are found on the epithelial sheet, converge to a single 'target' in the OB (Mombaerts, *et al.*, 1996). These targets are the glomeruli, spherical conglomerates of neuropil some 50–100 μm in diameter that consist of the incoming axons of olfactory sensory neurons and the dendrites of the main projection cells in the bulb, the mitral cells. The mitral cells,

together with the tufted cells, are the output neurons of the OB, and convey information to higher order olfactory structures. The relay from the nose to the mitral and tufted cells is strongly regulated by local intrabulbar circuitry.

Higher order olfactory structures targeted by the mitral and tufted cells include, from rostral to caudal, the olfactory peduncle (anterior olfactory nucleus, AON), piriform cortex (PC), olfactory tubercule (OT), entorhinal cortex (Ent), and some amygdaloid nuclei.

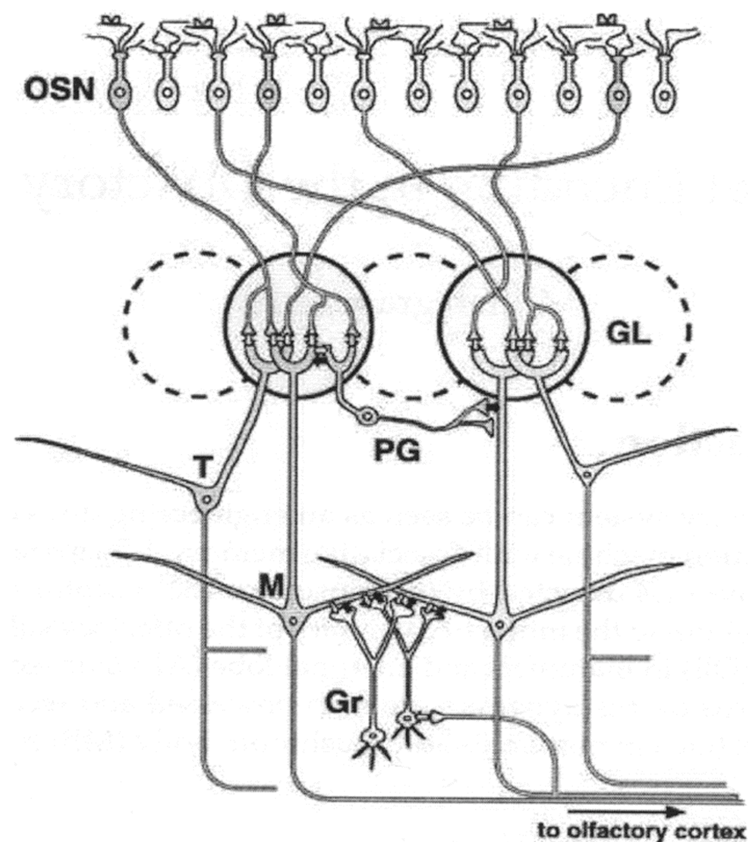

Fig 1.1. Schematic representation of the main olfactory system connections. Olfactory sensory neurons convey the sensory signal to the mitral cells of the olfactory bulb. The mitral cell axons project to the higher brain olfactory structures (olfactory peduncle, piriform cortex, olfactory tubercule, entorhinal cortex). OSN: olfactory sensory neurons. GL: glomerular layer. PG: periglomerular layer. T: tufted cells. M: mitral cells. Gr: granule cells. Figure adapted from Firestein, 2001

1.2. Organization of the main OB

The OB, the first relay station of the main olfactory system, has a characteristic laminar organization.

The most superficial layer of the OB is the Olfactory Nerve Layer (ONL), which contains thin and unmyelinated axons from the primary olfactory neurons, and olfactory glial cells that are generated during development from the olfactory epithelium and that migrate towards the OB.

Immediately deeper to the ONL is the glomerular layer (GL). The glomeruli (about 2000/ 3000 per bulb for rabbits and mice) are composed of neuropil-rich spheroid structures surrounded by a distinctive shell of glial cells and small neurons, periglomerular (PG) cells. Periglomerular cells are GABAergic inhibitory interneurons, with many small dendrites forming spine appendages with the dendrites of mitral and tufted cells. The olfactory sensory neurons project into the glomeruli, where they form synapses with the dendrites of the mitral and tufted cells.

Immediately deeper to the glomeruli is a layer with a relatively low cell density but a very dense neuropil, the external plexiform layer (EPL). The predominant neural elements in this layer are the dendrites of mitral/tufted cells. The EPL contains also the cell bodies of the tufted cells, projection neurons whose dendrites lie within the glomeruli and the axons of which project to the internal plexiform layer.

Deeper to the EPL is the mitral cell layer (MCL), which contains the big cell bodies of the mitral cells, arranged almost in a monolayer. Mitral cells, the principal output cells of the bulb, have one apical dendrite that enters one single glomerulus where it branches, and several secondary dendrites, which form several dendro-dendritic synapses with granule cells. The axons of mitral cells project to higher regions of the brain.

Subjacent to the mitral cell layer is the internal plexiform layer, containing many axons and dendrites but with a low density of cells, and deeper to it lies the granule cell layer. Granule cells are small GABAergic interneurons that possess many short dendrites forming dendro-dendritic synapses with mitral and tufted cells. Granule cells of the mammalian bulb exist in at least two populations, although the exact details of these populations may vary with species. One population of granule cells has been found with maximal spine density in the upper two sub-layers of the EPL,

while other granule cells have maximal spine density in the two lower sub-layers of the EPL. In the rat these two groups of granule cells were reported to correlate with the positions of the cell bodies. Thus, Orona et al. (1983) propose that there may be two parallel and partially independent olfactory processing systems in the bulb: the mitral cells and their associated granule cells, and the tufted cells, with their separated population of granule cells. My thesis work focuses on the mechanisms underlying the maturation of a subset of granule cells.
The OB serves different functions. First of all, it receives sensory information from the olfactory sensory neurons and it relays this to higher order olfactory structures; this function is served by the projection neurons, mitral and tufted cells. At the same time the bulb provides a first modulation of the sensory inputs, through the GABAergic inhibitory interneurons, which form dendro-dendritic synapses with the projection neurons at two anatomically different levels: the periglomerular interneurons and the granule cells.

The location and interconnections of interneurons (periglomerular and granule cells) in the OB are summarized in fig.1.2. On the basis of their neurotransmitter content, several interneuron subtypes can be distinguished in the OB. The most common type of interneuron contains GABA as its main neurotransmitter. GABA interneurons can be further subdivided on the basis of their content of calcium-binding proteins (calbindin, calretinin and parvalbumin) and neuropeptides (for example, neuropeptide Y). In addition, some interneurons of the OB and striatum do not use GABA as their principal neurotransmitter, but instead use dopamine or acetylcholine (Gall et al., 1987). GABA and tyrosine hydroxilase (TH), the dopamine-synthesizing enzyme, were found to coexist in a large number of neurons in the glomerular, granule and external plexiform layer. For instance, all the TH-immunoreactive periglomerular neurons also contained GABA, while there was an additional number of GABA-immunoreactive periglomerular cells (27%) which did not contain TH (Gall et al., 1987). Further studies indicated that heterogeneous chemical nature of olfactiory bulb interneurons corresponds to differences in dendritic arborization and synaptic contacts. For example, in the periglomerular layer TH+ interneuron processes form more synaptic terminals with the olfactory neuron afferences than the calbindin+ and calretinin+ interneurons (Kosaka et al., 1996; Toida et al., 1998).

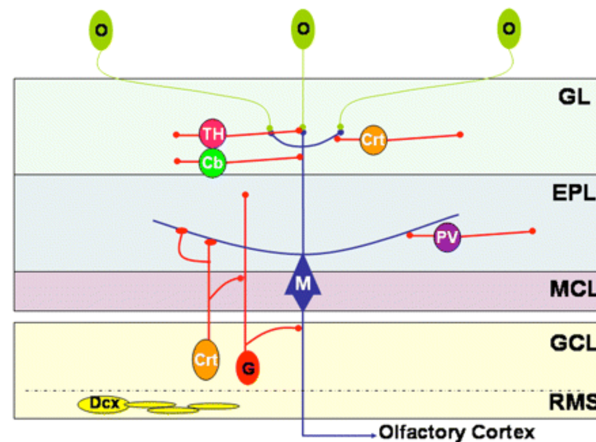

Fig. 1.2. Location and connections of interneurons in the olfactory bulb. The diagram represents a coronal section through an adult mouse olfactory bulb. New neuroblasts (Dcx positive) enter the bulb from the rostro-migratory stream, migrate radially and differentiate as interneurons in (i) the granule cell layer (GCL), (ii) the mitral cell layer (MCL), (iii) the external plexiform layer (EPL) and (iv) the glomerular layer (GL). Immunolabeling of Calretinin (Crt), Calbindin (Cb), Tyrosine Hydroxylase (TH) or Pavalbumin (PV) identifies subpopulations of olfactory interneurons distributed throughout the bulb as shown. G= granule cells. M= mitral cells. Figure from Young et al., 2007.

1.3. Development of the main OB

The development of the mouse main OB can be divided into two steps. First, evagination of the primordial OB from the telencephalon takes place on embryonic day 12.0–13.0. Second, from E12.5-13.0 the bulb primordium starts being populated by projection neurons and interneurons that have two origins: (i) a local origin within the OB and (ii) a subpallial origin in the subventricular zone of the lateral ganglionic eminence (LGE). The interneurons are continuously generated in the LGE and travel through the rostral migatory stream (RMS) into the OB throughout the life of the animal.

While the OB projection neurons, mitral and tufted cells, are only generated during embryonic life, a peculiarity of the OB interneurons (together with hippocampal granule cells) is that they are continuously generated during postnatal life and adulthood. Therefore the emphasis of investigation has traditionally been set on the generation of interneurons in the adult stage, and the embryonic stage has been comparatively neglected.

1.3.1 The developing subpallium

The developing telencephalon is anatomically divided into pallium (or developing cortex) and subpallium. The subpallium, the source of OB and other interneurons, comprises the medial and the lateral ganglionic eminences. Three main tangential pathways of migrating interneurons expressing the *Dlx* transcription factor originate from the subpallium: the latero-caudal migration stream that extends from the medial ganglionic eminence (MGE) to the cortex, the rostro-migratory stream that extends from the LGE to the OB and the latero-caudal migration stream that extends from the basal telencephalon to the striatum (Fig.1.3A). In the telencephalon, therefore, tangential migration is the primary mechanism by which interneurons reach their final position.

Distinct telencephalic progenitor zones are characterized by regionally restricted expression of transcription factors. The MGE expresses *Nkx2.1*, the MGE and LGE express *Gsh2* and *Mash1*, the corticostriatal sulcus expresses *Dbx1* and the developing cortex (pallium) expresses *Emx1* and *Pax6* (Fig.1.3B).

The rostro-migratory stream (RMS) contains migratory neuroblasts that travel in chains from the ganglionic eminences to the OB. Upon arrival in the OB, they migrate radially and become mature interneurons that populate the glomerular layer and the granule cell layer. In the adult, the subpallial ventricular zone which gives rise to the rostral migratory stream has been extensively characterized. It is known to be heterogeneous and contains three cell types: the slowly dividing stem cells (or B cells) expressing GFAP and astroglial markers, which are precursors for the transit-amplifying progenitors that express *Mash1* and *Olig2* (C cells). The migratory neuroblasts express neuronal markers such as ß-III-tubulin and PSA-NCAM, and are found in both the sub-ventricular zone (SVZ) and the RMS.

Although little is known about the molecular control of this process, a number of transcription factors have recently been shown to label specific subpopulations of the SVZ/RMS. For example, *Dlx2* labels both C cells and migratory neuroblasts, whereas *Olig2* and *Mash1* just label transit-amplifying (C) cells. None of these transcription factors remain expressed in the mature interneurons of the OB. However, other markers like *Pax6, Er81, Arx, Sp8, Dlx5* are expressed in the progenitor sub-ventricular zone, in the migrating neuroblasts of the rostro-migratory stream, and

remain expressed in the mature neurons once integrated in the OB periglomerular or granule layer.

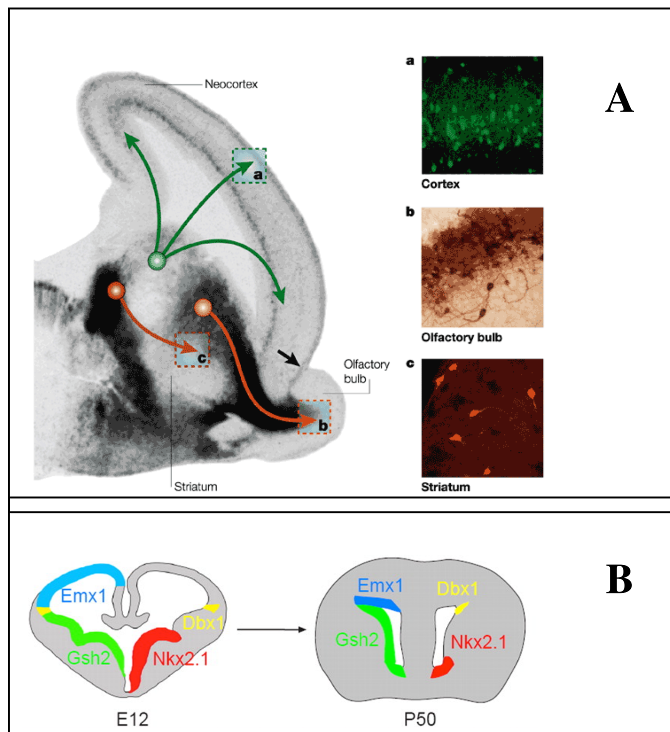

Fig. 1.3 (A) The three main telencephalic tangential migration pathways. Sagittal section of a mouse telencephalon at E15.5, which shows *Dlx 5/6* expression. The three main tangential pathways identified in this region are: (i) the latero-caudal migration from the subpallial telencephalon to the cortex (green), (ii) the medio-rostral migration from the subpallial basal telencephalon to the olfactory bulb (orange), and (iii) the latero-caudal migration from the basal telencephalon to the striatum (red). These migratory pathways give rise to a variety of interneurons shown in panels a, b, c. **(B)** Schematic showing the different embryonic neuroepithelial domains of the telencephalon and their relative contribution to generating the adult SVZ. (Marin et al, 2001)

1.3.2. The developing lateral ganglionic eminence

In 2003 Stenman et al. identified two distinct progenitor populations within the lateral ganglionic eminence (LGE). The first population, *Dlx5/6+/Isl1+*, migrates to the striatum, while the second population, *Dlx5/6+/Er81+*, situated in the dorsal most portion of the LGE, comprises OB interneuron progenitors that will form the rostral migratory stream and gives rise to precursors of the granule and periglomerular cell layer.

Other loss-of-function and lineage-tracing studies confirmed that the earliest population of OB interneurons during embryonic life originates from a restricted progenitor zone in the dorsal most part of the LGE (dLGE) (Wichterle et al., 2001; Yun et al., 2001). This progenitor population of pioneering OB interneurons expresses also markers such as *Sp8, Pax6, Arx* and *Dlx5/6*. The expression of those markers is maintained in the RMS and in mature OB interneurons. Loss of the corresponding genes results in a defect of OB granule and periglomerular cell formation.

Birth dating studies showed that OB interneuron precursors in the LGE are positionally specified and autonomously acquire a distinct migratory capacity between E12.5 and E14.5 (Tucker et al., 2006). The position in the dLGE confers LGE precursors with the unique ability to migrate to the OB and acquire characteristics distinctive of OB interneurons. Explantation and in vitro essays suggested that cell autonomous identity is reinforced by local signals, and is required for LGE cells to migrate selectively to the OB. This selective capacity is established during a critical period between E11.5 and E12.5 in the LGE. The nature of the local signals that allow the migration of the pioneer interneurons is not clear, yet.

1.3.3 Embryonic versus adult neurogenesis

Previous studies showed that embryonic and early postnatal neurogenesis from the dLGE gives rise to a population of OB neurons that has different characteristics from the neurons generated during adulthood (Lemasson et al., 2005). First, early born interneurons are specifically targeted to the external edge of the granule cell layer, while the newly born neurons are positioned deeper in older mice. Moreover, early-generated neurons survive till adulthood, while younger interneurons are replaced in a few weeks time. This suggests that the early born interneurons could be functionally different from the interneurons born during adulthood.

1.3.4 Regulation of OB interneuron differentiation

Although the mechanisms of regulation of OB interneuron differentiation have not yet been elucidated, molecules, in particular transcription factors, that control their specification, differentiation and migration are beginning to be identified.
Gsh1 and *Gsh2* homeobox genes are among the earliest markers of ventricular zone progenitors that produce telencephalic GABAergic neurons. These genes are required to produce OB interneurons through specifying progenitor domain identity and proper patterning of the telencephalon. Lack of *Gsh2* results in profound defects in telencephalic development, loss of markers for early development of the LGE and a delay in the appearance of GABAergic interneurons of the OB (Corbin et al., 2000).
Mash1 encodes a basic helix-loop-helix transcription factor that is also expressed in the subpallial ventricular zone, in which it is required for controlling the balance

between early and late progenitors and for the efficient generation of GABAergic neurons (Casarosa et al. 1999, Yun et al., 2002).

The *Dlx1,2,5,6* homeobox genes begin to be expressed after *Gsh1,2* and *Mash1* (Yun et al, 2002, 2003). *Dlx2* is expressed in a subset of ventricular zone cells; all four *Dlx* genes are strongly expressed in the sub-ventricular zone, and subsets of *Dlx* genes are expressed in differentiating forebrain GABAergic and dopaminergic neurons (Eisenstat et al., 1999; Long et al., 2003). Postnatally, *Dlx2* and *Mash1* are expressed in the adult sub-ventricular zone and rostral migratory stream in transient amplifying cells and neuroblasts. *Dlx1/2* -/- mice lack all OB GABAergic and dopaminergic interneurons (Bulfone et al., 1998), whereas *Dlx1*-/-, *Dlx2*-/- or *Dlx5*-/- mutants have less severe OB phenotypes (Qiu et al., 1995; Long et al., 2003).

Evidence was provided to show that the *Mash1* and *Dlx1/2* transcription factors have complementary roles in regulating *Notch* signaling, which mediates the temporal control of LGE neurogenesis (Yun et al., 2002).

Pax6 has an early role in patterning the OB anlage (Nomura et al. 2004, 2006); moreover, it acts with an opposite role together with *Gsh2* to control the dorsal-ventral identity of the telencephalon, specifying the boundary between pallium and subpallium (Toresson et al., 2000). *Pax6*, in addition, has a later role in generating dopaminergic periglomerular interneurons and is implicated in regulating development of superficial granule cells (Kohwi et al., 2005)

The *Sp8* zinc-finger is expressed in GABA positive and calretinin positive periglomerular interneurons, and it is required for their specification (Waclaw et al., 2006). *Er81*, an Ets transcription factor, shares the expression pattern with *Sp8* in the dLGE and RMS, and is also expressed in the deep granule cell layer, but its function in these cells is unknown.

1.3.5 Guidance of migration to the olfactory bulb

Little is known about the control of migration of progenitor interneurons to the OB. Slit ligands and the ROBO receptor are currently considered to be among the most important regulators of the migration. Slit proteins have been shown to repel OB interneurons (Chen et al., 2001; Sawamoto et al., 2006); there is also evidence that a SLIT gradient within the lateral ventricles guides interneuron migration toward the OB; furthermore, *Slit1/2* -/- mutants have a small OB (Sawamoto et al., 2006).

Neuregulin signaling is another important regulator of OB interneuron migration, because *ErbB4* -/- mutants have altered neuroblast chain organization and migration (Anton et al., 2004), as well as a deficit in the placement and differentiation of OB interneurons. Signaling by the secreted protein PK2 attracts OB interneurons, as PK2 mutants show defects in the organization of the OB and rostral migratory stream (Ng et al., 2005). Secretion of Reelin from mitral cells is implicated in the signaling that guides tangential migration of OB interneurons (Hack et al., 2002).

Cells that migrate in the rostral migratory stream express high levels of the polysialylated neural cell-adhesion molecule (PSA-NCAM). Genetic deletion of PSA-NCAM disrupts neuronal migration to the OB. However, cells that lack PSA-NCAM migrate normally along the RMS of wild-type mice, indicating that PSA-NCAM is an essential component of the migration substrate (Hu et al., 1996).

1.4. The *Teashirt* genes

1.4.1 Discovery of the *Drosophila Teashirt*

The *Drosophila Teashirt* gene was discovered in 1991 through screening of a large number of random integrations of a reporter allele in the genome (Fasano et al, 1991). The reporter allele, when integrated into the *Teashirt* locus, was expressed during embryogenesis in the thoracic part of the trunk, in the future anus, and in internal tissues such as a portion of the midgut, the central and peripheral nervous system and the somatic mesoderm.

Sequencing of the *Teashirt* cDNA revealed a putative protein of 993 amino acids, containing three uncommon zinc finger motifs ($CX_2CX_{12}HX_5H$), in which the two-histidine residues are separated by five amino acids instead of three. Relatively few zinc finger proteins contain a HX_5H motive. Some examples are Hunchback, Pep and the Suvar(3)7 proteins.

A second unusual characteristic of the Teashirt protein is that the fingers are widely spaced (89 and 44 amino acids, respectively), instead of being clustered as is often the case with previously described zinc finger proteins. In *Drosophila*, Hunchback and Pep are examples of zinc finger proteins containing isolated fingers.

Mutations of *Drosophila Teashirt* disrupt the entire trunk domain, showing that the gene is required for normal development of the ventral trunk region of embryos,

which correlates with the spatial expression of the gene along the anterio-posterior axis but not in the dorso-ventral axis.

1.4.2. *Drosophila Teashirt*: a homeotic gene modulating the *Wingless* signaling pathway.

Several lines of evidence showed that *Teashirt*, whose transcripts always accumulate in segments destined to acquire trunk identities, is a homeotic gene. Mutations in a homeotic gene preserve the normal number of trunk elements, but change their identity.

In 1992 Röder et al. showed that when *Teashirt* is missing, the ventral part of the trunk is transformed to anterior head identity, and a similar phenotype is provoked by lack of the classical known homeotic genes *Antennapedia* and *Bithorax*. The same study showed, using analysis of double mutant phenotypes, that *Teashirt* gene activity is required for the normal function of the *Antp* and *BX-C* genes, and the synergistic function of these genes represses anterior head development and head gene activity in the trunk. Ectopic misexpression of *Tsh* during larva development induces transformation of the labial to prothoracic segmental identity, while it has no effect on the identity of the trunk segments where it is normally expressed (De Zulueta et al., 1994). Moreover, monitoring of *Teashirt* transcription using an enhancer gene trap line inserted in the gene locus showed a positive autoregulation of the endogenous *Teashirt* gene (Robertson et al., 2004).

More recent data further explained the axial patterning mechanism in the larva, showing that HOM-C and HOX proteins, responsible for the trunk patterning, are expressed under the regulation of a network of zinc-finger proteins, such as disconnected (disco) and Teashirt.

In addition to the *Homeotic* (*Hox*) genes, a second class of genes called "segment – polarity genes" contributes in the larva to determine differences in the epidermis in each segment. Segment polarity genes encode for components of the Hedgehog and Wingless signal transduction pathways, and are responsible for intersegmental differences of identity. The *Wingless* (*wg*) gene, analogous to the vertebrate *Wnt*, encodes a secreted glycoprotein. A conserved signal transduction pathway transmits the signal promoted by W*g* following its secretion. At the end of this pathway, two proteins are responsible for Wg output: Armadillo (Arm; similar to vertebrate b-

catenin) and the *Drosophila* T-cell factor (dTcf), analogous to the vertebrate T-cell factor (Tcf/LEF). Both Arm and dTcf seem to be crucial for transmission of all known effects of *Wg* on transcription. *Teashirt* was shown to bind Arm in order to modulate trunk-specific *Wg* output. In each trunk segment, higher levels of *Teashirt* are present in the nuclei of *Wg*-active cells than in non-active ones; high *Teashirt* levels suffice to elicit a *Wg*-like response (Gallet et al., 1998). Subsequently, it was shown that *Wingless* signaling promotes phosphorylation and nuclear accumulation of *Teashirt* protein (Gallet et al., 1999); *Teashirt* binds to the C-terminal of *Arm* and it is in a complex with *Shaggy* (*Sgg*, homologous to the vertebrate glycogen synthase kinase 3b), allowing the regulation of the *Wg* target genes specific to the trunk.

1.4.3 Vertebrate hortologues to *Drosophila Teashirt*

Two murine and two human orthologues of the *Drosophila Teashirt* gene were first described in 2000 by Caubit and colleagues, who showed that the vertebrate and *Drosophila Teashirt* proteins share a conserved structural organization. The proteins possess three atypical (Cx2Cx12HMx4H) widely spaced zinc finger motifs and an acidic domain. To date only 15 known sequences encode proteins containing such zinc finger motifs. The conservation between *Drosophila* and murine Teashirt (Tshz) amino acid sequences is low (35%) and it is essentially restricted to the region of the atypical zinc finger motifs and the acidic domain. *Drosophila* and vertebrate Teashirt proteins show the highest conservation in the first and second zinc finger motif: 52% identity and 65% similarity. The murine Teashirt proteins possess two classical (Cx2Cx12Hx3-4H) zinc-finger motifs at their C-terminal end which are not found in *Dtsh*. A third vertebrate hortologue, *Teashirt3* (*Tshz3*), was detected to be expressed in a temporal and spatial pattern that is similar, though not identical, to *Tshz1* (L.F., N. Core´ and X.C., unpublished).

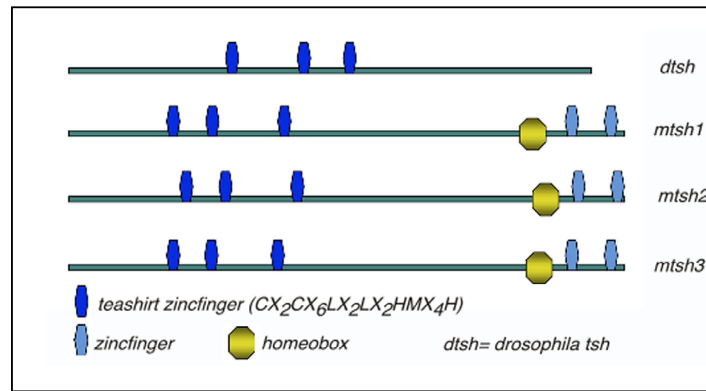

Fig.1.4. Schematic representation of the structural organization of *Drosophila* and murine *Teashirts*

Tshz1 expression is first detected in E9 – E9.5 embryos (20-22 somite stage) and transcripts are only detected in the trunk region. At this stage, *Tshz1* is expressed in the dorsal part of the neural tube, the dorsal part of the somites, the mesenchyme of the developing forelimb buds and in the region of the foregut. In E11 embryos, *Tshz1* expression is maintained in the dorsal part of the neural tube and in the dermomyotome, and is also detected in the first and second branchial arches and in the limb bud (Long et al., 2001).

Tshz2 mRNA can be first be detected in the presumptive forelimb buds of E9-9.5 embryos. At E10, strong expression is observed in the limb buds and in somites. In the limb bud at E13.5, *Tshz2* mRNA is localized in interdigital regions.
This data, published in 2001 by Long and colleagues, are in agreement with the results of the expression analysis that I performed at early developmental stages (Fig. 1.5).

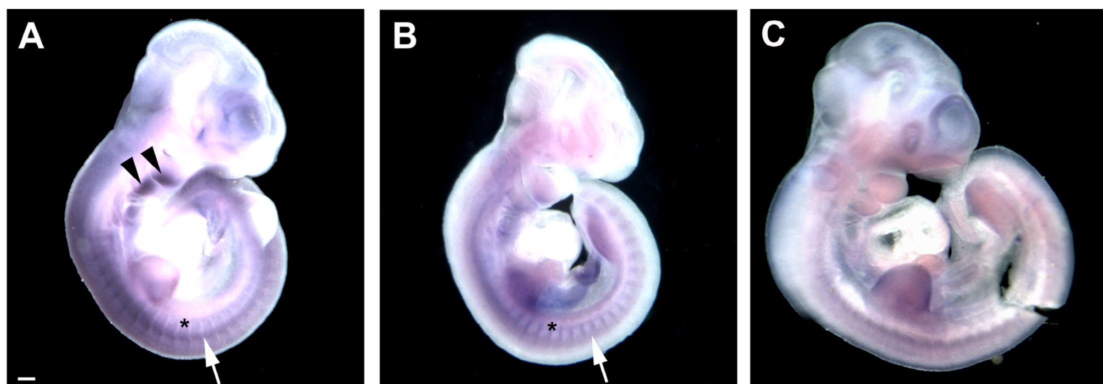

Fig.1.5. Whole-mount in situ hybridization of *Tshz1* (A), *Tshz2* (B) and Tshz3 (C) at E10.5. (A) Expression of *Tshz1* is detected in branchial arches (black arrow heads), in the ventral spinal cord (white arrow) and the caudal and rostral myotome (asterisk); (B) *Tshz2* expression is detected in the ventral spinal cord (white arrow), in the caudal myotome (asterisk) and gut primordium. (C) Expression of Tshz3 is weak at E10.5. Bar: 200 µm.

1.5 The aim of the project

The aim of my PhD project is to research the functions of murine *Teashirt* genes in the developing central nervous system. *Tshzs* were found to be expressed in the postnatal dorsal spinal cord by genome-wide expression analysis, which suggested a possible function in the central nervous system. Moreover, expression analysis in the developing dorsal spinal cord revealed strong *Tshz1* and *Tshz2* expression throughout embryonic development, starting at E10.5.

In order to investigate a possible function in the development of the nervous system, I established two strains of mutant mice where respectively *Tshz1* and *Tshz2* coding sequences were disrupted by insertion of a Gap43-GFP cassette. As *Tshz1* mutant mice died a few hours after birth, I generated a *Tshz1* floxed allele in embryonic stem cells to obtain a conditional mutation of the gene. *Tshz2* mutant mice were viable and appeared normal.

A preliminary functional analysis of *Tshz1* in the developing spinal cord was undertaken using genome-wide expression analysis in the dorsal spinal cords of E15.5 control and mutant mice. The results showed minimal variations of gene expression in mutant compared to control dorsal spinal cord. In addition, histological and marker analyses revealed no major changes in the appearance of the spinal cord. Possibly, the three *Tshz* genes expressed in similar patterns cooperate during spinal cord development.

However, *Tshzs* are expressed in non-overlapping patterns in the olfactory bulb (OB). Therefore, I focused my analysis of *Tshz1* mutant on the OB in the expectation that *Tshz1* functions would not be compensated by other *Tshzs*: *Tshz1* is expressed in the granule cell layer, *Tshz2* just in the mitral cell layer and *Tshz3* in the accessory OB. Thus, I was able to define a function of *Tshz1* in maturation and correct radial migration of granule cell precursors in the postnatal OB.