3. GENETICS OF EARLY NEUROGENESIS IN DROSOPHILA

3.1. Introduction

Two major processes govern the development of nervous systems: cell-autonomous determination linked to cellular division ('mosaic development') and non-autonomous processes depending on cell-cell interactions ('regulation'). While in simple nervous systems like in C. elegans rigid determination prevails as a mechanism (chapter 12), neuronal fate in higher species is strongly influenced by signals from the cellular environment. Most often, a combination of determinative and regulative processes is operating, as illustrated by early neurogenesis in insects. Most of what is known about these mechanisms derives from studies in Drosophila.

3.2. Early steps in the development of the central and peripheral nervous system

Neurogenesis in Drosophila is a biphasic process consisting of an embryonic and a postembryonic period of neurogenesis. During embryogenesis primary neurons are generated that become functional in the larval central nervous system (CNS). Subsequently, during postembryonic neurogenesis secondary neurons are generated, which build up the adult brain during larval and pupal stages. During embryonic stages neural precursor cells or neuroblasts (NBs) segregate from the procephalic and ventral neuroectoderm by a process called lateral inhibition. Subsequently NBs divide asymmetrically in a stem-cell like fashion thereby self-renewing and producing smaller ganglion mother cells (GMCs). The GMCs have a limited mitotic potential and divide only once more to generate a pair of neurons and/or glial cells.

At the end of embryogenesis NBs undergo a quiescent phase and only a subset of NBs enter mitosis again after larval hatching to generate secondary neurons for the central brain and the ventral nerve cord ganglia. The visual anlagen of the adult brain derive from optic lobes of embryonic origin. During larval stages neuroepithelial cells of the optic lobes proliferate through symmetric cell division and transform later into asymmetrically dividing NBs to generate the neurons for the different optic ganglia.
Sensory cells and associated structures of the peripheral nervous system (PNS) derive from 'sensory mother cells' (SMCs), which segregate in the embryo from fields of the lateral ectoderm. Again, SMCs are singled out by processes of lateral inhibition. In simple bristle sensilla SMCs divide twice forming a sensillum that consists of 4 cells:

- sensory cell (mechanosensory)
- thecogen cell (glial sheath)
- trichogen cell (bristle forming cell)
- tormogen cell (socket forming cell)

Internal sensilla, such as 'chordotonal' stretch receptor organs, consist of 4 homologous cells that also derive from two successive SMC divisions. Adult external and internal sensilla develop essentially from imaginal discs, but otherwise the underlying cellular and genetic processes are comparable to those operating during embryogenesis.
3.3. Proneural genes commit groups of cells towards the neural fate

The cascade of genes that control neurogenesis has been studied mainly in imaginal discs. In a first step, 'proneural genes' of the achaete-scute complex (AS-C) make groups of cells competent to become neurons, i.e., they fix the future position of sensilla. The AS-C includes 4 complementation groups:

- achaete (ac)
- scute (sc)
- lethal of scute (l'sc)
- asense (ase)

Internal chordotonal organs depend on another proneural gene, atonal.

Deletion of the entire AS-C results in the loss of all external sensilla. Mutations in certain AS-C genes remove particular sensilla, depending on which genes are affected. ac and sc are expressed in individual, overlapping patterns and regulate each other. Their
functions are partially redundant. Proneural genes are transcription factors encoding a basic helix-loop-helix (bHLH) domain, which allows homo- and heterotypic dimerization and DNA binding. Such dimers can act either as activators or repressors. Heterodimers are for example formed with another crucial bHLH factor, Daughterless (Da). Loss-of-function mutants of this gene lack all SMCs. The spatiotemporal expression pattern of proneural genes is regulated by other transcription factors.

3.4. Neurogenic genes control neural precursor numbers through lateral inhibition
Proneural genes usually generate an excess of cells committed to the neural fate. However, as soon as a developing NB or SMC has acquired neural competence, it prevents neighboring cells from adopting a neural fate through a process of lateral inhibition. Neighboring cells will eventually become epidermal precursors. Lateral inhibition is controlled by a group of genes called ‘neurogenic genes’. Defective lateral inhibition in neurogenic mutants results in neural hypertrophy in the CNS or an increase of sensilla at the expense of epidermis. The precise spacing of hairs and bristles on the insect cuticle depends strongly on the action of neurogenic genes. Two different processes are involved in lateral inhibition: long range signalling via diffusible factors and direct cell-cell communication. Neurogenic genes are controlling only in the latter process.

**Examples of neurogenic genes:**
- Delta (Dl)
- neuralized (neu)
- Notch (N)
- kuzbanian (kuz)
- mastermind (mam)
- Enhancer of split (E(spl))

Neurogenic genes are arranged in an interacting (‘epistatic’) genetic chain, E(spl) acting most downstream. Epistatic relations can be studied by rescue experiments whereby it is tested whether a particular neurogenic gene can reverse a mutant phenotype of another neurogenic gene. The establishment of an epistatic chain does predict a genetic interaction but not the molecular nature of interactions, i.e., whether they act at the transcriptional, translational or protein level.

To study which of the neurogenic genes are acting in the signal sending cell (ligand) and which genes are acting in the signal receiving cell (receptor) marked undifferentiated cells from neurogenic regions of diverse mutants were transplanted to similar wildtype regions. For example, if mutant cells are able to form epidermal cells in wildtype tissue, their receptor for the inhibitory signal must be intact. Hence, the mutant acts on the sender side. It turned out that for example N and E(spl) are on the receptor side (cell autonomous), while other genes such as Delta and Neur have a role in the signal sending cell.
Notch and Delta are transmembrane proteins with extracellular domains partially homologous to vertebrate epidermal growth factor (EGF). These two proteins were shown to form heterophilic interactions and act mainly between neighbouring cells. Delta is expressed high in the presumptive NBs and signals to Notch receptors expressed in the presumptive epidermal precursors. EGF-repeats and a specific ‘DSL’ (Delta-Serrate-Lin) binding region on the ligand were found in a variety of receptor-ligand systems of diverse functions. Intracellular domains of Di-like ligands are short, whereas those of N-like receptors are very large and include signals for nuclear transport.

a) Wing blade of a wild-type Drosophila melanogaster (left), and a mutant with a partial loss of the Notch gene (right) (photographs by Mark Fortini). b and c) Drosophila has one Notch receptor (dNotch) and two transmembrane bound ligands for Notch, named Delta (Di) and Serrate (Ser). (from Radtke and Raj, 2003)
In more recent studies it was discovered that in the signal sending cell the ligand Delta interacts with the E3-ubiquitin ligase Neuralized (Neur) (blue) for ubiquitylation and activation. Delta is then competent to bind to the Notch receptor and induces signalling. Prior to Neur interaction, Delta is inactive and might be endocytosed and degraded.

In the signal receiving cell the Notch receptor (red) is produced in the endoplasmatic reticulum (ER) where it interacts with the O-fucosyl transferase (O-Fut; light brown) and is transported to the Golgi. In the Golgi Notch is processed by the Furin-like convertase (lilac) and glycosylated by glycosyltransferases such as O-Fut and Fringe (dark brown). Notch is transported to the cell membrane where it can interact with its ligand Delta (green). Notch binding to the ligand Delta leads to two proteolytic cleavages of the Notch receptor. In Drosophila the metalloprotease Kuzbanian (blue) catalyses the S2-cleavage and generates a substrate for S3-cleavage by the γ-secretase complex. The proteolytic cleavage releases the Notch intracellular domain (NICD), which enters the nucleus and interacts with the transcription factor Suppressor of Hairless (Su(H) ) (green). Upon binding, the repressive Su(H) complex recruits co-factors such as Mastermind (Mam) (orange) and transforms into an activator complex that induces the expression of Notch target genes (Enhancer of Split genes). In the signal receiving cell Delta activation can be inhibited by Bearded proteins (orange), which bind and inhibit Neur (blue) mediated ubiquitylation.
3.5. Regulation of Delta and Notch activity
Initially, all cells committed to the neural pathway by proneural genes express N and Dl. How is a given cell then selected to become a NB or a SMC, i.e., why does it upregulate Dl and downregulate N? Probably a slightly higher Dl expression in one cell is sufficient to induce downregulation of proneural genes (and indirectly of Dl) in the neighboring cell. Thus, by 'negative feedback' an initially small asymmetry may develop into a stable difference. However, purely arbitrary competition between equivalent cells is hardly compatible with the stereotyped arrangement of bristle sensilla in the epidermis. It remains possible that early asymmetries are genetically fixed. Asymmetric distribution of gene products may be generated during cell division, a process that occurs for example in asymmetric dividing NBs and SMCs (see Chapter 8).

As discussed above Delta in one cell can activate Notch in its neighboring cells, which is called trans-activation. However, more recently it has been discovered that high Delta levels in the same cell can also bind to Notch and inhibit signaling. This is a mechanism called ligand-mediated cis-inhibition. Cell culture experiments and computational models, which integrate trans-activation and cis-inhibition led to a better understanding of lateral inhibition. The models can explain how neighboring cells can rapidly become either the signal-sending or the signal-receiving cell.

There is cross-regulation of Notch signaling with many other signaling pathways. The Wingless signaling pathway was identified as a negative regulator of the Notch signal transduction cascade. Wg belongs to a group of secreted growth factors. It is for example expressed in a single row of cells on the wing margin, the only wing region that gives rise to sensory bristles. Wg binds to the membrane receptor, Dfz2, which then activates a cytoplasmic protein Dishevelled (Dsh). Dsh is believed to interact with Su(H)/CSL to reduce their level within the active transcription factor pool. Hence, a cell activated by Wg is committed to a neural rather than epidermal fate. Both the Notch cascade and its control by Wg are involved in a variety of developmental processes and the mechanism of Wg-Notch signaling crosstalk seems to be evolutionary conserved.

3.6. Proneural and neurogenic genes in other phyla
The Notch signaling pathway is evolutionary highly conserved from invertebrates to higher mammals. N homologs with similar functions as in Drosophila are known in C. elegans. A number of proneural and neurogenic genes have been identified in zebrafish, Xenopus, chicken, mouse and humans. Examples are homologs of Notch, Delta, E(spl) and Su(H). Sometimes the function is difficult to test in vertebrates because different copies of proneural and neurogenic genes often have partially redundant functions. Yet, knock-out mice, injection of sense- or antisense RNA in Xenopus oocytes, or the incorporation of gene constructs with retroviral vectors suggest that the processes in early vertebrate neurogenesis are very similar as observed in the fly. For example, constitutively active N mutants also suppress the neurogenic phenotype, and Dl homologs act as neuron-specific signaling molecules. In general
Notch signaling maintains the undifferentiated or neural stem cell state and inhibits premature neuronal differentiation in many neural systems.

### 3.7. Neuronal identity genes
A series of neural specific genes downstream of neurogenic genes are involved in the control of neuronal or sensillum identity. An early gene in this cascade is *cut*, which commits SMCs towards the bristle fate rather than the chordotonal fate (see Chapter 3.2.). Other genes, like *numb* and *oversensitive* interact with the two successive divisions of SMCs, resulting in only neural cells (*oversensitive*) or in only non-neural cells (*numb*). Another neuronal identity gene *pox-neuro* controls the segregation between mechanosensory and gustatory bristle sensilla.

### 3.8. Specification of temporal identity
During neural development neural progenitors generate distinct cell types over time. Populations of such multipotent progenitors are found in the vertebrate cortex, in the retina and the spinal cord. One big question is what are the extrinsic and intrinsic factors that regulate neuronal diversity during development. As discussed earlier *Drosophila* neuroblasts produce near invariant lineages, usually consisting of distinct cell types. In addition, neuroblasts generate their progeny cells in a stereotype birth order. Hence, *Drosophila* neuroblasts provide a good model system for studying how the temporal identity of neural precursors is regulated to generate neuronal diversity.

(Pearson and Doe, 2004)

<table>
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<th>Temporal identity changing intrinsic cues</th>
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<td>Spatial identity</td>
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<td>Cell diversity</td>
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(Pearson and Doe, 2004)

There are two different ways for regulating temporal identity:

(a) Intrinsic regulation of temporal identity: Here, the progenitor acquires initially a unique spatial identity that is based on its anterior-posterior and dorso-ventral position within the developing nervous system. The progenitor then becomes independent on spatial patterning cues and produces a stereotype cell lineage
over time. The composition of the lineage is therefore strictly based on progeny birth order and not affected by environmental changes (intrinsic cues).

(b) Extrinsic regulation of temporal identity: Here, the progenitor also acquires a spatial identity on the basis of its anterior-posterior and dorso-ventral position within the developing nervous system. However, progenitor identity then changes in response to alteration in the environment (extrinsic cues).

\[ \text{(Pearson and Doe, 2004)} \]

\[ \text{In vitro isolation and transplantation experiments can provide evidence for one or the other model to specify temporal progenitor identity. } \]

\[ \text{Drosophila neuroblasts faithfully recapitulate their } \textit{in vivo} \text{ division pattern } \textit{in vitro}, \text{ and to date only intrinsic factors have been implicated in the regulation of their temporal identity. } \]

\[ \text{The majority of embryonic neuroblasts sequentially express the transcription factors Hunchback (Hb), Krüppel (Kr), Pdm and Castor (Cas). GMCs and their neuronal progeny maintain the expression of the transcription factor that is present in the neuroblast at the time of the GMC’s birth. These leads to a layered expression of these temporal transcription factors with Hb expression in deep layer neurons and Kr, Pdm and Cas in progressively more superficial layers. The expression correlates with birth order and not with a particular cell type. For instance Hb positive progeny cells are all early born but depending on the neuroblast lineage can give rise to either a motor neuron or a glial cell. Loss of Hb in the embryonic CNS leads to a loss of first-born GMCs and the corresponding progeny due to cell death, fate skipping, or fate transformation leading to a duplication of the next-born identity. In contrast continuous misexpression of Hb leads to supernumerary progeny cells that adopt a first-born fate. Similarly, in the absence of Kr second-born cell types are missing and the forced expression of Kr in neuroblasts can again lead to extra cells, which adopt the second-born fate. It was also shown that the neuroblast looses competence to generate early born progeny cells in response to Hb expression. Furthermore, postmitotic neurons cannot be transformed any longer to an early born fate.} \]
A temporal order of progeny cells has also been observed in the vertebrate retina. In chick, *Xenopus* or fish ganglion cells, horizontal cells, cones and amacrine cells seem to differentiate first whereas bipolar rods and Müller glia differentiate last. However, more recent clonal analysis and mathematical modeling suggest that retinogenesis in vertebrates follows a stochastic model, in which division mode and cell fates cannot be predicted by birth order only.

![A Stochastic RPC Clone](image)

(Chen et al., 2012)

4. GENETICS OF NERVOUS SYSTEM REGIONALIZATION

4.1. Introduction
4.2. Dorso-ventral body axis inversion between insects and vertebrates
4.3. Cephalic gap genes in embryonic brain development of flies and mice
4.4. Homeotic genes pattern the posterior brain in flies and mice

4.1. Introduction
In terms of neuroanatomy and development, the brains of insects and vertebrates look strikingly different. In insects, the CNS is generated by the proliferation of neuroblasts, which delaminate from a ventral neuroectoderm. In mammals, in contrast, the CNS derives from the neural tube that invaginates from a dorsal neuroectoderm. This suggests that these two brain types derived independently during evolution. Recent work has led to the discovery of a number of evolutionarily conserved control genes implicated in embryonic brain development. Both the expression domains and mutant phenotypes of these genes reveal a high degree of homology from vertebrates to insects. Mutations in these genes lead to similar brain phenotypes in the two phyla,
such as the absence of large neurogenic regions. These data suggest conserved functions of these genes in the two types of brains.

4.2. Dorsoventral body axis inversion between insects and vertebrates
In classical embryology, the opposite location of the nerve cord in insects (ventral) and the spinal cord in vertebrates (dorsal) has been taken as major evidence for an independent evolution of these two types of CNSs. However, new developmental, genetic and molecular data in *Drosophila*, frogs, zebrafish and mouse, have provided strong support for an inversion of the dorsoventral (D/V) body axis in insects and vertebrates, and thus for a common evolutionary origin of the two phyla and their nervous systems. Support for this hypothesis is provided by the analysis of two antagonistically acting signaling systems that control D/V axis formation. One of them is represented by the *decapentaplegic* (*dpp*) gene in *Drosophila* and its vertebrate homolog *BMP4* (*Bone Morphogenetic Protein 4*). Interestingly, though, the site of action of the Dpp/BMP4 proteins is localized to the dorsal side in insects and to the ventral side in vertebrates. Conversely, the antagonistically acting extracellular signaling proteins Short gastrulation (*Sog*) in the fly and the homologous vertebrate Chordin protein act from ventral and dorsal, respectively. In both phyla, it is the region where *sog/Chordin* is expressed – and thus dpp/BMP4 signaling is inhibited, which later gives rise to the neuroectoderm. The fact that homologous signaling proteins, engaged in similar molecular interactions, lead to the initial induction of the ventral neurogenic region in insects and the dorsal neurogenic region in vertebrates strongly supports the idea of a homology of the CNS in these animal groups.

Another set of genes implicated in D/V patterning also manifest a D/V inversion of their relative expression domains in insects and vertebrates. In *Drosophila*, the key players are the homeobox genes *vnd*, *ind* and *msh*, which are involved in the formation and specification of neuroblasts in the ventral, intermediate and dorsal columns of the neuroectoderm, respectively. Again, the homologous vertebrate genes *Nkx2*, *Gsh* and *Msx* are also involved in D/V patterning, but their expression domains are inverted.

Alternative explanations to the D/V inversion hypothesis cannot be ruled out. However, independent support for a common evolutionary origin of the CNS of insects and vertebrates is provided by considering the key control genes which are involved in antero-posterior (A/P) patterning of the developing brain ( 4.3.-4.4.).

4.3. Cephalic gap genes in embryonic brain development of flies and mice
As in the trunk region, the segment-polarity genes *engrailed* and *wingless* are expressed in the head of *Drosophila*. Also, the A/P organization of the head seems to be controlled by gap genes, even though ‘cephalic’ gap genes manifest certain unique features. The expression domains of two of these, *orthodenticle* (*otd*) and *empty spiracles* (*ems*) are determined by the maternally derived Bicoid gradient, as for normal gap genes, and they recognize the compartment boundaries defined by *engrailed*. The
expression patterns of *otd* and *ems* strongly overlap, in contrast to the non-overlapping expression stripes of gap genes in the trunk. Loss-of-function alleles of *otd* and *ems* lead to specific brain deletions, indicating that these genes are required in early specification of the anterior brain anlage. Interestingly, the mouse homologs of *otd* and *ems*, *Otx1/2* and *Emx1/2*, respectively, are also involved in anterior brain patterning.

Embryonic expression of *otd* in *Drosophila* is restricted to most of the protocerebral primordium and the anterior deuterocerebral primordium. Intriguingly, in *otd/otd* mutant embryos, these anlagen are deleted. The phenotype is due to defective specification of the neuroectoderm resulting in the absence of neuroblasts in the affected region. It is correlated with the loss of expression of the proneural gene *lethal of scute* (*l'sc*) (⇒ 3.3.), which is known to induce neurogenic competence. Overexpression of transgenic *otd* in *otd* null mutant embryos is able to rescue the missing tissue. Ubiquitous overexpression of *otd* in the wildtype can lead to the induction of ectopic brain structures. These structures express a protocerebrum-specific gene indicating that *otd* is able to provide protocerebral identity.

In the mouse, *Otx2* is expressed during gastrulation in cells that are involved in the induction of the anterior neural plate, as well as in the responding cells in the anterior neuroectoderm. Later, *Otx2* expression extends throughout most of the prosencephalic and mesencephalic neuroectoderm with its posterior border at the midbrain-hindbrain boundary. Accordingly, *Otx2* appears to have two functions, the induction of the anterior neural plate and the specification of forebrain and midbrain areas. This is confirmed by rescue experiments in chimeric mice. In homozygous mutant *Otx2* embryos, the rostral

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Expression domains and null mutant phenotypes of the *otd/Otx2* genes in *Drosophila* and mice. b1-3, protocerebrum, deuterocerebrum, tritocerebrum; D, diencephalon; M, mesencephalon; r1-8, rhombomeres 1-8; s1-3, mandibular, maxillary & labial neuromeres; Sc, spinal cord; T, telencephalon; VNC, ventral nerve cord (from Sprecher & Reichert, 2003)
neuroectoderm is not specified and, in consequence, these mutants die early in embryogenesis. Expression of \textit{Otx1} occurs later than that of \textit{Otx2}. \textit{Otx1} null mutant mice are viable, but 30\% of them die within the first postnatal month. These mice show a reduced thickness of the telencephalic cortex, due to a lack of cell proliferation. Experiments replacing \textit{Otx2} with \textit{Otx1} indicate that \textit{Otx1} can rescue the inductive function of \textit{Otx2}, but not its later requirement for specification of forebrain and midbrain areas.

Expression of the \textit{ems} gene in \textit{Drosophila} is found in two stripes in the anterior deuto- and tritocerebral primordia. As in the case of \textit{otd/otd}, \textit{ems} loss-of-function mutants lead to severe deletions of these anlagen, due the absence of neuroblasts (correlated with loss of expression of \textit{l'sc}). Rescue of the null mutant phenotype can be achieved by ubiquitous overexpression of \textit{ems} at stage 11. In the developing mouse neocortex, \textit{Emx2} is expressed in a gradient, with high caudomedial and low rostrolateral expression levels. As one might expect, \textit{Emx2} null mutant mice show a reduction of caudomedial areas and an expansion of rostrolateral areas.

Taken together, the expression domains of the \textit{otd/Otx} and \textit{ems/Emx} gene families in the developing brain are remarkably similar in flies and mice. Furthermore, mutant analysis suggests that the corresponding genes might perform comparable functions in insects and vertebrates during early development of the anterior brain. The most reasonable explanation for these findings is that expression and function of these genes in brain embryogenesis are evolutionarily conserved.

To confirm this hypothesis, cross-phylum rescue experiments were performed in which the mutated endogenous gene of interest was replaced by a homologous, functional vertebrate or insect gene. Ubiquitous overexpression of the human \textit{Otx2} gene (less efficiently for \textit{Otx1}) under the control of an inducible promoter was able to rescue the gap-like anterior brain deletion in \textit{otd} null mutant fly embryos. Also, cross-phylum rescue experiments in the mouse demonstrated that \textit{otd} can restore many brain defects caused by an \textit{Otx1} null mutation, such as a full rescue of cortex formation. Cross-phylum rescue experiments were also carried out successfully for the \textit{ems/Emx2} genes. Ubiquitous overexpression of \textit{Emx2} rescued the brain phenotype of \textit{ems} null mutant flies (lack of deuto- and tritocerebrum). In summary, the cross-phylum rescue experiments confirm that the functions of the \textit{otd/Otx} and \textit{ems/Emx} genes in brain development are to a large degree evolutionarily conserved.

4.4. Homeotic genes pattern the posterior brain in flies and mice

Homeotic (Hox) genes are found throughout the animal kingdom and are essential for patterning the A/P body axis. Since Hox genes are expressed also in the developing CNS (except in the most anterior brain region), they might control its A/P specification in a similar way as for the rest of the body. In both insects and vertebrates, Hox genes show a spatial co-linearity in their expression patterns, i.e, more 3' located genes in the chromosome are expressed more anteriorly along the body axis than more 5' located genes. This applies essentially to the five anteriorly expressed genes \textit{labial (lab)}, \textit{proboscipedia (pb)}, \textit{Deformed (Dfd)}, \textit{Sex combs reduced (Scr)}, \textit{Antennapedia (Antp)} of
Drosophila and their mouse homologs Hoxb-1, Hoxb-2, Hoxb-4, Hoxb-5 and Hoxb-6. However, in contrast to the perfect spatial co-linearity of Hox gene expression in the epidermis, pb and its mouse homologue Hoxb-2 is expressed in the CNS more posteriorly than lab/Hoxb-1. In the developing vertebrate CNS, Hox genes are expressed in the hindbrain and spinal cord. Patterning domains show a two-rhombomere periodicity of expression starting at rhombomere 3.

In the fly, loss-of-function mutations for lab and Dfd have been shown to result in severe defects of the embryonic brain. In lab null mutants, defects are observed in the posterior part of the tritocerebrum, which is the normal expression domain for lab. Interestingly, the affected mutant domain is still present and the neuroblasts giving rise to this area are correctly located. However, their progeny remain undifferentiated and do not express neuronal markers indicating that these cells fail to adopt a neuronal fate. Comparable defects are observed in Dfd null mutants for the mandibular neuromere, the normal expression domain for Dfd.

Comparison of Hox gene expression domains and mutant phenotypes in the CNS of Drosophila and the mouse. (A) In lab null mutants, cells of the posterior part of the tritocerebrum (b3) are correctly located, but fail to assume neuronal fate. (B) Double mutant Hoxa-1 / Hoxb-1 embryos result in a reduced size of r4 and a loss of expression of r4-specific markers (from Sprecher & Reichert, 2003)
Strikingly, the *lab* null mutant phenotype can be functionally rescued by almost any Hox gene. Yet, more 3' located Hox genes have higher rescue efficiency than more 5' located ones. Hence, even though the amino acid sequence of the fly Hox proteins has diverged considerably, most of them can still functionally replace the *lab* protein.

In the mouse, neuroanatomical analysis of Hox mutants reveals that these genes act also in specific domains along the A/P neuraxis. Loss-of-function mutations of the *lab* ortholog *Hoxa*-1 result in segmentation defects in the mutant domain of the hindbrain, such as a reduced size of rhombomeres 4 and 5. However, the normal identity of r4 is not altered. Inactivation of *Hoxb*-1, another *lab* ortholog, does not change the size of r4, but results in a loss of identity of this area leading to a partial transformation of r4 to r2. Double knock-out of *Hoxa*-1 and *Hoxb*-1 causes a reduced size of r4 and additionally a loss of expression of r4-specific markers, resulting in the formation of a domain of unknown identity. These findings suggest that r4 identity is achieved by the synergistic action of *Hoxa*-1 and *Hoxb*-1, and point to a surprisingly similar role of these genes as for the orthologous gene *lab* in the posterior tritocerebrum of the fly.

Thus, for the Hox genes, as for the cephalic gap genes, we are confronted with homologous developmental control genes that have strikingly similar expression patterns and functional roles in brain development in insects and mammals. Taken together, these data imply that these basic molecular genetic features of brain development have been conserved through evolution.

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Chapter 4