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Spatially Restricted Patterns of Gene Expression Define Potential Boundaries in the Developing Mouse Forebrain

by

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THESIS

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Introduction

The functional diversity of the central nervous system relies on regional differences in the identity, organization and properties of its nerve cells. Despite exponential progress in studies of developmental neurobiology, the mechanisms that control regional specification and differentiation remain unknown. Recent efforts have attempted to demonstrate analogies in pattern specification between vertebrates and the better studied invertebrate systems. These studies rely heavily on the idea that the embryonic CNS is segmented; that is, populations of cells comprise repeating units along the antero-posterior axis. The ultimate identities of each segment would be uniquely specified by the expression of regulatory genes, analogous to the manner in which *Drosophila melanogaster* develops. Cellular differentiation and migration, as well as neuronal connectivity could then generate the region-specific tissues of the brain. Selector genes may define the fate of cells in the vertebrate systems as they do in *Drosophila*, and the study of their expression patterns might suggest functions of these genes and ought to provide molecular markers for descriptive analysis. It is probable that homeobox, *Wnt* and other classes of genes are some of the candidates responsible for the regulation of neural development. Genes in these classes have been shown to be expressed in temporally and spatially restricted patterns in the developing nervous system, and their expression is consistent with a role in the development of particular neural structures.

One of the difficulties in determining the exact contribution of these developmental regulators in neural development is the paucity of information on the organization of the forebrain. Studies in the last century have described the early stages of forebrain organization in a neuromeric theory (von Kupffer, 1906; Rendahl, 1924; von Haller, 1929; Bergquist and Kallen, 1954,1955; Coggeshall, 1964; Kallen, 1965; Vaage, 1969; Keyser, 1972; Gribnau and Geijsberts, 1985; Altman and Bayer, 1986, 1988; Puelles et al., 1987, 1991, 1992). Originally, the neuromeric theory relied on

morphological data to distinguish segmental relationships within the diencephalon and the telencephalon, for example, the presence of transverse constrictions that subdivided the neural tube into repeating neural segments or neuromeres. Later, genetic cell fate determinants were postulated to subdivide the neural tube into discrete transverse and longitudinal domains that develop along distinct pathways. Evidence of genetic markers and cell fate determinants that delineate the boundaries between the neuromeres may provide molecular evidence for this theory. Each neuromere would have elements in common with other neuromeres in addition to unique genes that control development in specific regions. Conclusive evidence for a segmental organization of the forebrain will require the identification of common repeated elements in each transverse compartment.

Studies on *Drosophila melanogaster* strongly suggest that genetic mechanisms control the specification of different regions of the invertebrate body plan, where the longitudinal axis of the embryo is divided into segments by the serial expression of the gap, pair-rule and segment polarity genes (Levine and Harding, 1989). Expression of the homeotic genes (McGinnis and Krumlauf, 1992) distinguish the arrangement and patterns of the elements within a segment. The identified products of the homeotic genes contain the homeodomain motif (Gehring, 1987) and are thought to be transcriptional regulators. Regional differentiation of the vertebrate nervous system may use similar mechanisms as those that mediate *Drosophila* development. For example, the mouse *Hox* genes, homologs of the *Drosophila Antennapedia-Bithorax* complex (Acampora et al., 1989; Duboule and Dolle, 1989; Graham et al., 1989; Kappen et al., 1989), are expressed in overlapping, though not identical, domains along the A-P axis in vertebrate embryos. The anterior boundaries of individual *Hox* genes are found at proposed neuronal segment boundaries in the mouse hindbrain (Hunt et al., 1991). Positional information is also specified in *Drosophila* by secreted proteins such as *wingless* (*wg*) (Baker, 1987; Rijsewijk et al., 1987). The *Wnt* gene

family (MaMahon et al., 1992; Nusse and Varmus, 1992), the vertebrate homologs of the *wg* gene, also play a role in the development of discrete regions of the CNS (e.g. the targeted disruption of the *Wnt-1* gene in mouse results in a loss of the midbrain in homozygous animals (McMahon et al., 1992)). Expression of the *wg* protein in *Drosophila* may also affect the expression of other homeobox genes, like *engrailed* (Heemskerk et al., 1991). It is possible that these gene families, among others, may interact in the regulation of vertebrate brain development.

Studies of the forebrain have lagged behind progress in the more caudal regions of the CNS for two reasons. First, the complex morphology and histology of the forebrain has made it a difficult structure to study. Furthermore, the identification of candidate regulatory genes have only recently been described. Several families of transcription factors genes, such as homeobox (e.g.: *Dlx, Dbx, Gbx, Gtx, Nkx, Otx* , *Pax, POU* and *Emx*) and helix-loop-helix (e.g: *myc, Id, Sim*) genes, and several growth/differentiation factor genes, including members of the *Wnt* gene family (e.g.: *Wnt-3, Wnt-3a, Wnt-5a* and *Wnt-7b*) have been found to have restricted patterns of expression in the embryonic mouse forebrain.

Results from this and other laboratories show that several genes that have been implicated in the regulation of embryonic development are expressed in spatially and temporally restricted transverse and longitudinal domains within the forebrain, consistent with a neuromeric model of the forebrain. Bulfone et al. (1993) developed a model of forebrain organization at E12.5 in the mouse based on the existence of developmental compartments arranged as segments with both alar and basal components. These experiments did not identify molecular markers for each of the hypothesized domains, and so a search was initiated for other genes that recognize the compartments predicted by the model. In addition, studies of gene expression in the forebrain at E10.5 were conducted to determine whether the expression patterns observed at E12.5 were constant or developmental age-dependent. Despite the

complexity of the patterns of gene expression provided by the markers that were chosen, all those studied do consistently respect the proposed longitudinal and transverse boundaries proposed in the neuromeric model of Bulfone et al. (1993). The expression patterns of these genes suggest that the forebrain is segmented, and that, based on the discrete neural structures whose morphological boundaries these genes help define, they play an integral role in defining the identity of specific neural tissues.

Materials and Methods

Mouse embryos: Timed-pregnant BALB/C mice were obtained from Simonsen Laboratories, Gilroy, CA and timed-pregnant CD-1 and DUB mice were obtained from Roger Pederson's laboratory. The day on which a copulatory plug was found was considered E0.5. The mothers were killed by cervical dislocation and the embryos were isolated. Embryos aged E12.5 and older were immediately frozen in 2-methylbutane cooled to the temperature of dry ice. These embryos were stored at -80 degrees C until they were used for cryostat sectioning. Embryos aged E9.5 through E11.5 were fixed in 4% paraformaldehyde in 1X PBS for 6 hours, allowed to sink through 30% sucrose overnight and shaken in 30% sucrose:OCT compound (1:1) for two hours before embedding in OCT compound and cryostat sectioning.

The isolation of the *Dlx-2 (Tes-1)* clone was described by Porteus et al. (1991, 1992). For these experiments, a subclone containing 730 bp from the 3' untranslated region was used to generate probes.

The isolation of the *Gbx-2* clone was described by M.A. Frohman and G.R. Martin (unpublished results). A *Gbx-2* riboprobe was transcribed from a 650 bp fragment derived from the 3' untranslated region of the *Gbx-2* cDNA clone.

The isolation of *Wnt-3a* was described by Roelink and Nusse, 1991. A *Wnt-3a* riboprobe was transcribed from a 700 bp fragment derived from the noncoding region of the *Wnt-3a* cDNA clone.

The isolation of *Wnt-5a* was described by McMahon et al., 1992. A *Wnt-5a* riboprobe was transcribed from a 360 bp fragment derived from the noncoding region of the *Wnt-5a* cDNA clone.

The isolation of *Wnt-7b* was described by McMahon et al., 1992. A *Wnt-7b* riboprobe was transcribed from a 300 bp fragment derived from the 3' noncoding region of the *Wnt-7b* cDNA clone.

^{35}S-labeled riboprobes were made according to the procedure of Zoeller et al. (1989). The amount of radioactivity incorporated into acid precipitable counts using 1 ug of DNA template was between 1 and 3 X 10^7 cpm The *in vitro* transcription reaction was carried out as follows: One ug of Proteinase K-treated, linearized plasmid was incubated with 10 U of T3 or T7 RNA Polymerase (Stratagene) in 40 mM Tris-HCl pH 8.0, 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl, 30 mM dithiothreitol, 1.25 mM each rATP and rGTP and 12.5 uM each rCTP and rUTP, 50 U RNase Block (Stratagene) at 37 degrees C for 30 minutes. The incubation was then repeated, under the same conditions, adding another 10 U of RNA Polymerase. The reaction was completed by an incubation with 10 U of RNase-free DNase (Stratagene) and 10 U of RNase Block (Stratagene) at 37 degrees C for 10 minutes. This reaction was followed by an ethanol precipitation in 20 mM EDTA pH 8.0, 400 mM LiCl, and 1 mg/ml yeast tRNA. The riboprobes were dissolved in 100 ul DEPC-treated water and 10 U of RNase Block (Stratagene) and stored at -80 degrees C.

In situ hybridizations: In situ RNA hybridization and autoradiography with ^{35}S labeled riboprobes were carried out as described by Porteus et al. (1992). The results were analyzed using light field and dark field microscopy.

Results

E10.5

Expression pattern of Dlx-2 in the forebrain

At E10.5, *Dlx-2* is expressed in the brain, the branchial arches and the limbs of the developing mouse embryo (Bulfone et al., 1993b and Figure 1). Within the forebrain, the caudal zone of expression is confined to the ventral thalamus, the posterior entopeduncular area, the hypothalamic cell cord and the suprachaismatic area (SCH). Expression stops at the boundary between the SCH and the preoptic area. The rostral zone of expression extends from the preoptic area along the base of the cerebral vesicle under the primordia of the lateral (LGE) and medial ganglionic eminence (MGE). Expression continues rostrally along the septal area, but ends just before the primordia of the cerebral cortex.

Expression pattern of Gbx-2 in the forebrain

At E10.5, *Gbx-2* appears to be expressed in the spinal cord and in the forebrain (Figure 1). Within the forebrain, *Gbx-2* has two discrete domains of expression: the dorsal thalamus and the basal telencephalon. As in E12.5, the domain of expression within the basal telencephalon is complementary with that of *Dlx-2*. *Gbx-2* is expressed in the rostralmost part of the MGE and the AEP.

E12.5

To compare directly the patterns of expression of the *Dlx-2*, *Gbx-2*, *Wnt-7b*, *Wnt-5a*, and *Wnt-3a* genes in the E12.5 mouse embryo, *in situ* RNA hybridization experiments were performed using serial sagittal and coronal sections. These experiments have largely been published, but were repeated to determine whether their boundaries of expression fit with the neuromeric model proposed. The expression data

is shown in Figure 2. (Adjacent photos within a row represent adjacent sections hybridized to the genes of interest. Each successive row shows expression of these genes on progressively more posterior regions of the telencephalon).

Expression patterns of Dlx-2 in the forebrain

There are two major domains of *Dlx-2* expression in the forebrain, separated by a zone of tissue where there is no expression of this gene (Bulfone et al., 1993 and Figure 2). This is the same pattern that was observed at E10.5. In the telencephalon, the expression domain includes the medial ganglionic eminence (MGE), the lateral ganglionic eminence (LGE) and the septum (SE). The zone of expression extends caudally from the SE, LGE and MGE into a boundary domain in the stalk of the cerebral hemisphere. Here it is expressed in the anterior entopeduncular area (AEP) and the anterior preoptic area (POA).

The neuroepithelium of the cerebral cortex, the caudal ganglionic eminence (CGE) and the eminentia thalami (EMT) are essentially unlabelled. The *Dlx*-negative zone also includes the supraoptic/paraventricular area (SPV), the anterior hypothalamus (AH), and the posterior preoptic area (POP). These unlabelled regions are apparent in both the sagittal (not shown) and the coronal sections (Figure 2).

Adjacent to the *Dlx*-negative region, there is a second domain that strongly expresses *Dlx-2.* The region of expression extends from the posterior preoptic area (POP) to the zona limitans intrathalamica. This longitudinal band of expression includes the suprachiasmatic area (SCH), the hypothalamic cell cord (HCC), the posterior entopeduncular area (PEP) and the ventral thalamus (VT). Therefore, the most caudal limit of *Dlx-2* expression is at the zona limitans. There also appears to be weak *Dlx-2* expression along the rostral part of the basal plate in the regions of the lateral tuberal hypothalamus (TU) and the mammilary area (MA). Strongest expression of *Dlx-2* in the wall of the neural tube is found in the subventricular zone.

Expression patterns of Gbx-2 in the forebrain

At E12.5 in the mouse, *Gbx-2* is expressed in four discrete domains: the spinal cord, the hindbrain, the caudal diencephalon and the basal telencephalon (Bulfone et al, 1993 and Figure 2). Within the forebrain, *Gbx-2* expression is in the prospective dorsal thalamus (DT), and is confined to the alar domain. The caudal boundary of *Gbx-2* expression is at the pretectum, whereas the rostral boundary abuts the zona limitans, which separates the dorsal and the ventral thalami. *Gbx-2* expression in the dorsal thalamus appears to abut, but not overlap, the domain of expression of *Dlx-2* in the ventral thalamus. *Gbx-2* expression in the walls of the diencephalon defines four zones: the ventricular zone (little or no expression), the subventricular zone (strong expression), the intermediate zone (weak expression) and the mantle (strong expression).

The second domain of *Gbx-2* expression in the forebrain is in the basal telencephalon. These *Gbx-2* positive cells are centered in the rostralmost part of the MGE and AEP, complementary to the expression pattern of *Dlx-2* in the basal forebrain. *Dlx-2* is expressed in the subventricular stratum of the MGE and AEP whereas *Gbx-2* is expressed in the adjacent mantle. It is possible that this *Gbx-2* expression is in the nucleus basalis, in which case acetyl cholinesterase coexpression could be used to identify these cells more accurately.

Expression pattern of Wnt-7b in the forebrain

The *Wnt-7b* expressing domain in the telencephalon is in postmitotic cells of the cortical plate (McMahon et al., 1992 and Figure 2). Migrating neurons in the intermediate zone appear to be *Wnt-7b* negative. The subplate appears clearly labeled in older sections (E14.5 and E16.5; Figure 3) extending into the olfactory bulb. This area will eventually develop into the cerebral cortex and other nuclear subcortical structures.

There are two other separate domains that are positive for *Wnt-7b* expression in the periventricular region. One of them extends from the rostral half of the midbrain to the

SCH. Alar expression within this domain defines a transverse boundary within the midbrain. Expression is continuous between the midline and the SCH along the ventralmost part of the alar plate. *Wnt-7b* extends uniquely into the dorsalmost alar region with expression into the caudal ganglionic eminence and archicortex, limiting with *Gbx-2* expression.

Expression pattern of Wnt-5a in the forebrain

Wnt-5a is primarily expressed in the facial area, although it is expressed in a few regions of the telencephalon (McMahon et al., 1992; and Figure 2). Most rostrally, it is expressed in the alar dorsal thalamus and in the choriodal epithelium, where its expression abuts that of *Gbx-2.* Further caudally, it is expressed in the basal pretectum and the rostralmost part of the basal midbrain. There is also some discrete expression in the hindbrain, along the alar region of the rhombomeres.

Expression pattern of Wnt-3a in the forebrain

At E12.5 in the mouse, *Wnt-3a* is expressed in very few detectable regions of the forebrain (Roelink and Nusse, 1991 and Figure 2). Within the telencephalon, Wnt-3a is detected only in the eminentia thalami and in the caudal archicortex.

E14.5, E16.5

Expression pattern of Gbx-2 in the forebrain at later developmental ages

Gbx-2 continues to be expressed in the same domains as at age E12.5 (E14.5 expression data shown in Figure 3). It is strongly expressed in the dorsal thalamus, where its expression boundaries abut the pretectum caudally and the zona limitans rostrally. Again, *Gbx-2* is complementary in expression to *Dlx-2* in the diencephalon, where the zona limitans separates the domains of expression of these two genes.

Discussion and Conclusions

I have described the expression patterns of several genes (*Dlx-2*, *Gbx-2*, *Wnt-7b*, *Wnt-5a*, and *Wnt-3a*) at different developmental ages (E10.5, E12.5, E14.5 and E16.5) in the mouse embryo. In all cases studied, the expression patterns of these genes define domains delineated by sharp boundaries. These boundaries are reproducibly observed at the same positions in each of several embryos.

At the expression boundaries, RNA transcripts from these genes are restricted to cells on one side of the boundary. Furthermore, some of the expression boundaries coincide with morphological structures, such as ventricular ridges and external furrows that are perpendicular to the longitudinal axis. The results described here support the hypothesis that the forebrain is organized into neuromeres, and these gene expression domains provide markers for this segmental organization. Because these genes encode transcription factors and secreted growth factors, it is possible that these genes are involved in the specification and differentiation of neuronal cell types and play a role in the definition of the proposed segments in the mouse forebrain.

Expression patterns of Dlx-2, Gbx-2 and Wnt-7b

Dlx-2 is a member of a homeobox gene family (Porteus et al., 1991; Price et al., 1991; Robinson et al., 1991), and is a homolog of the *Drosophila Dll* gene (Cohen et al., 1989). *Dlx-2* is expressed in two domains within the forebrain, separated by an optoeminential zone where it is not expressed. Within the wall of the neural tube, *Dlx-2* expression is limited to a subventricular layer, and within this layer, most cells express *Dlx-2*. Expression at E10.5 of *Dlx-2* and *Gbx-2* are the same as those observed at E12.5, suggesting that their expression is constant, and not developmental age-dependent.

For the most part, the regions of the brain that express *Dlx-2* are sharply delineated from those that do not. Thus, at these boundaries, cells that strongly express *Dlx-2* are

adjacent to cells that express less or no *Dlx-2.* These types of boundaries occur in several places. There is a sharp boundary near the sulcus between the lateral ganglionic eminence and the neocortical primordia. Also, caudally at the zona limitans, there is a sharp boundary of *Dlx-2* expression between the dorsal thalamus and the ventral thalamus. Unlike *Dlx-2*, *Gbx-2* is expressed primarily in the mantle, and not in the subventricular zone. There is little or no expression in cells adjacent to the ventricle. In the dorsal thalamus, it is expressed throughout the mantle, although it is expressed at higher levels in a deep and in a superficial mantle zone. These two zones may correspond to domains within the dorsal thalamus where distinct nuclei are developing.

Within the forebrain, the *Gbx-2* gene is related to the expression pattern of *Dlx-2* in two regions. There is a sharp border at the zona limitans between the expression of *Dlx-2* in the ventral thalamus and *Gbx-2* in the dorsal thalamus. *Gbx-2* is also expressed in the basal forebrain in a superficial mantle zone of the medial ganglionic eminence and adjacent anterior entopeduncular area. The *Gbx-2* positive cells occupy a small domain of more mature cells at the base of the eminence. While there appears to be no overlap of these genes at this border, there may be some mixing of *Dlx-* and *Gbx-2* positive cells at the boundaries of these regions.

In the forebrain, *Wnt-7b* is expressed within the cortex, as well as in several periventricular domains. Its expression abuts that of *Dlx-2* caudal to the lateral and medial ganglionic eminences. More caudally, its expression in the pretectum abuts that of *Gbx-2* in the dorsal thalamus and along the alar basal boundary of the dorsal thalamus.

Transverse and longitudinal domains within the forebrain

The prosencephalon, the rostralmost part of the neural tube, is divided into two major transverse domains: the diencephalon and secondary prosencephalon (Figure 4). The secondary prosencephalon consists of the telencephalic and optic vesicles, the

preoptic area and the hypothalamus. The prosomeric theory postulates the existence of both transverse and longitudinal domains that subdivide the embryonic forebrain into regions that follow discrete developmental paths. This theory takes into account the bending of the longitudinal axis at the cephalic flexure (Figure 5). The observation of ventricular ridges and external furrows in this region supports this theory. The work described herein supports the existence of these alar and basal longitudinal zones that span the brain and whose rostralmost limit is just behind the optic stalks. Thus, the longitudinal zones appear to be divided into transverse domains in both the diencephalon and the secondary prosencephalon (including the telencephalon). The expression patterns of the genes described here are consistent with this model of the organization of the forebrain (Figure 5).

The transverse zones of the forebrain have been named prosomeres. There are three diencephalic prosomeres and three secondary prosencephalic prosomeres (where p1 is the most caudal and p6 is the most rostral; see Figures 4 and 5). By this nomenclature, the alar p1 encompasses the pretectum, the alar p2 encompasses the dorsal thalamus, and alar p3 corresponds to the ventral thalamus, with these boundaries supported by the gene expression data presented here.

Transverse and longitudinal domains of expression in the diencephalon

The spatially restricted pattern of gene expression of *Dlx-2*, *Gbx-2*, *Wnt-7b*, *Wnt-5a*, *Wnt-3a* and others fit within the framework of the proposed longitudinal and transverse regions of the diencephalon (expression data schematically illustrated in Figure 6). The p2 segment is delineated by the expression of *Gbx-2*, whereas *Dlx-2* is expressed in p3-p6. In this way, the p1/p2 boundary is defined by the caudal limit of expression of *Gbx-2*, and the p2/p3 boundary (the zona limitans) is defined by the rostral limit of expression of *Gbx-2* and the caudal limit of expression of *Dlx-2*. The expression of *Wnt-7b* and *Gbx-2* define the p1/p2 boundary. The expression of other genes respect, if not define, these boundaries. These transverse zones also have the

morphological characteristics of neuromeres: ventricular ridges and external furrows are present at the boundaries between p1 (pretectum), p2 (dorsal thalamus) and p3 (ventral thalamus). Longitudinal zones in the forebrain can also be delineated by the expression patterns of these genes. For example, expression of *Dlx-2* and *Gbx-2* is restricted to the alar plate.

Transverse and longitudinal domains in the secondary prosencephalon

The organization of the secondary prosencephalon into transverse domains is uncertain because of the lack of clear morphological markers of segmentation. Three transverse domains within the secondary prosencephalon have been postulated: p4, p5, and p6. The expression of *Wnt-7b* and that of *Dlx-2* support the transverse boundary between p4 and p5 (Figures 2 and 5). Based on the expression pattern of these and various other genes published in the literature, the precise boundaries between p4 and p5 and between p5 and p6 have been proposed. Each of the genes considered respects spatially restricted boundaries of expression, and future studies will address the expression patterns of these and other genes at E12.5 and at different developmental ages.

List of Figure Legends

Figure 1. Comparison of *Dlx-2* and *Gbx-2* expression using *in situ* RNA hybridization to E10.5 parasagittal sections. *A-C* show cresyl violet-stained sections obtained from the same regions as the sections used for *in situ* RNA hybridization shown to their right. They were photographed using bright-field microscopy; *a-f* were photographed using dark-field microscopy. All of the sections were obtained from a single mouse embryo. *a-c* were analyzed for the expression of *Dlx-2*, *d-f* for the expression of *Gbx-2*.

Figure 2. Comparison of *Dlx-2*, *Gbx-2*, *Wnt-7b*, *Wnt-5a* and *Wnt-3a* expression using *in situ* RNA hybridization. D-F show cresyl violet-stained sections obtained from the same regions as the sections used for *in situ* RNA hybridization photographed using bright-field microscopy. *a-c* were analyzed for *Dlx-2* expression, *d-f* for the expression of *Gbx-2*, *g-i* for the expression of *Wnt-7b*, *j-l* for the expression of *Wnt-5a* and *m-o* for the expression of *Wnt-3a*. *a-o* were analyzed using dark-field microscopy. All of the sections were obtained from a single mouse embryo.

Figure 3. Comparison of *Dlx-2*, *Gbx-2* and *Wnt-7b* expression using *in situ* RNA hybridization to E14.5 parasagittal sections. *A-C* show cresyl violet-stained sections obtained from the same regions as the sections used for *in situ* RNA hybridization shown to their right. They were photographed using bright-field microscopy; *a-j* were photographed using dark-field microscopy. All of the sections were obtained from a single mouse embryo. *a-c* were analyzed for the expression of *Dlx-2*, *d-f* for the expression of *Gbx-2*, and *g-i* for the expression of *Wnt-7b*.

Figure 4. Realistic map of the expression patterns of *Dlx-2*, *Gbx-2* and *Wnt-3* (discussed in Bulfone et al., 1993b) genes in a schematic medial view of an E12.5 mouse brain. The transverse (neuromeric) subdivisions are delineated by solid lines that are perpendicular to the principal longitudinal subdivision that subdivides the alar and basal zones. This figure is included to show the principal anatomical regions of the mouse telencephalon upon which the neuromeric subdivisions are based.

Figure 5. Topologic map of the expression patterns of *Dlx-2*, *Gbx-2* and *Wnt-3* (discussed in Bulfone et al., 1993b) genes in the brain of an E12.5 mouse. In this figure, the longitudinal axis of the brain has been deconvoluted, and the transverse and longitudinal domains are indicated by black lines (the organization of the region rostral to the rhombencephalon is theoretical).

Figure 6. Schematic representation of the expression patterns of *Dlx-2*, *Gbx-2*, *Wnt-7b*, *Wnt-5a* and *Wnt-3a* in a scematic medial view of an E12.5 mouse brain.

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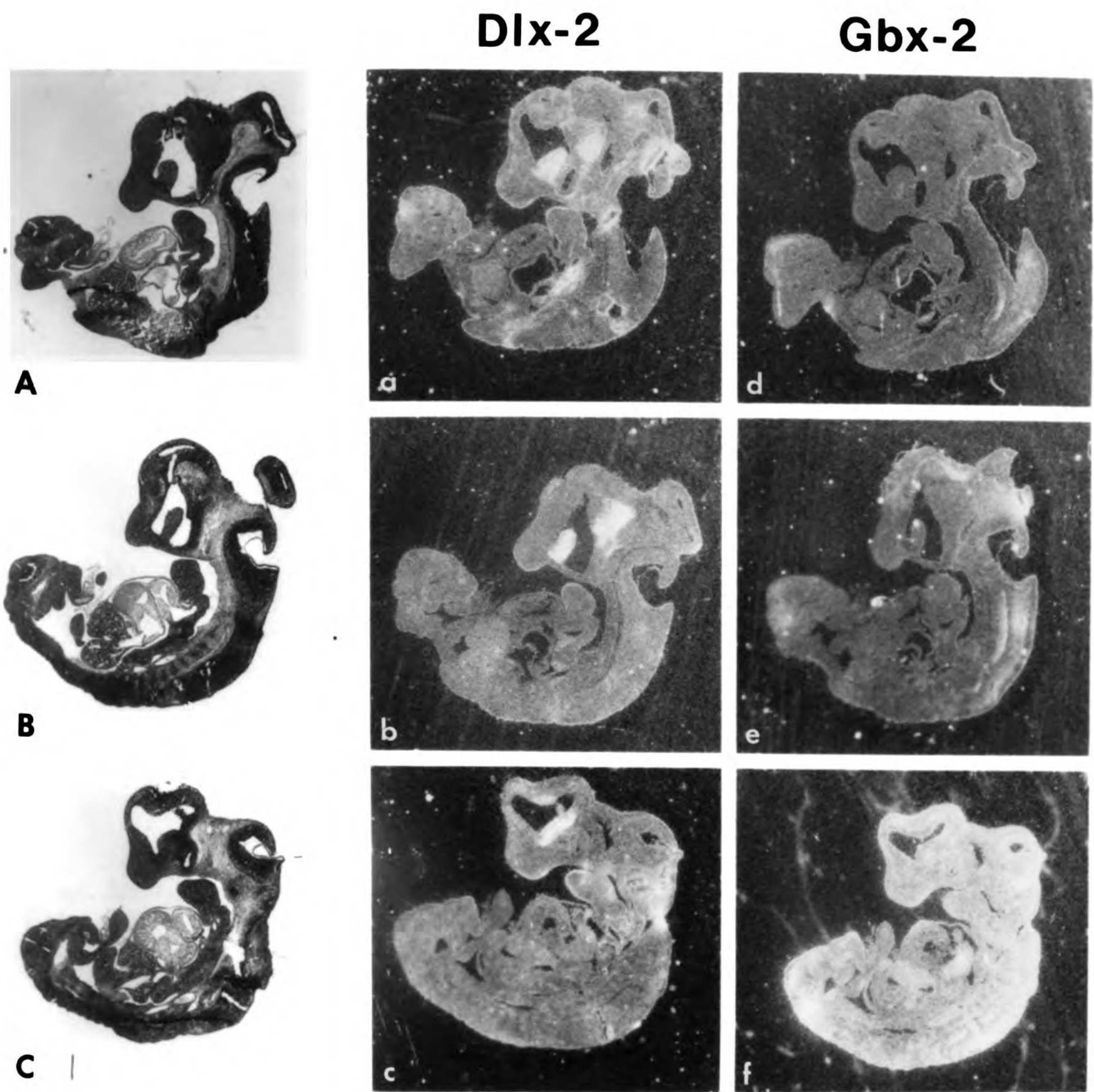
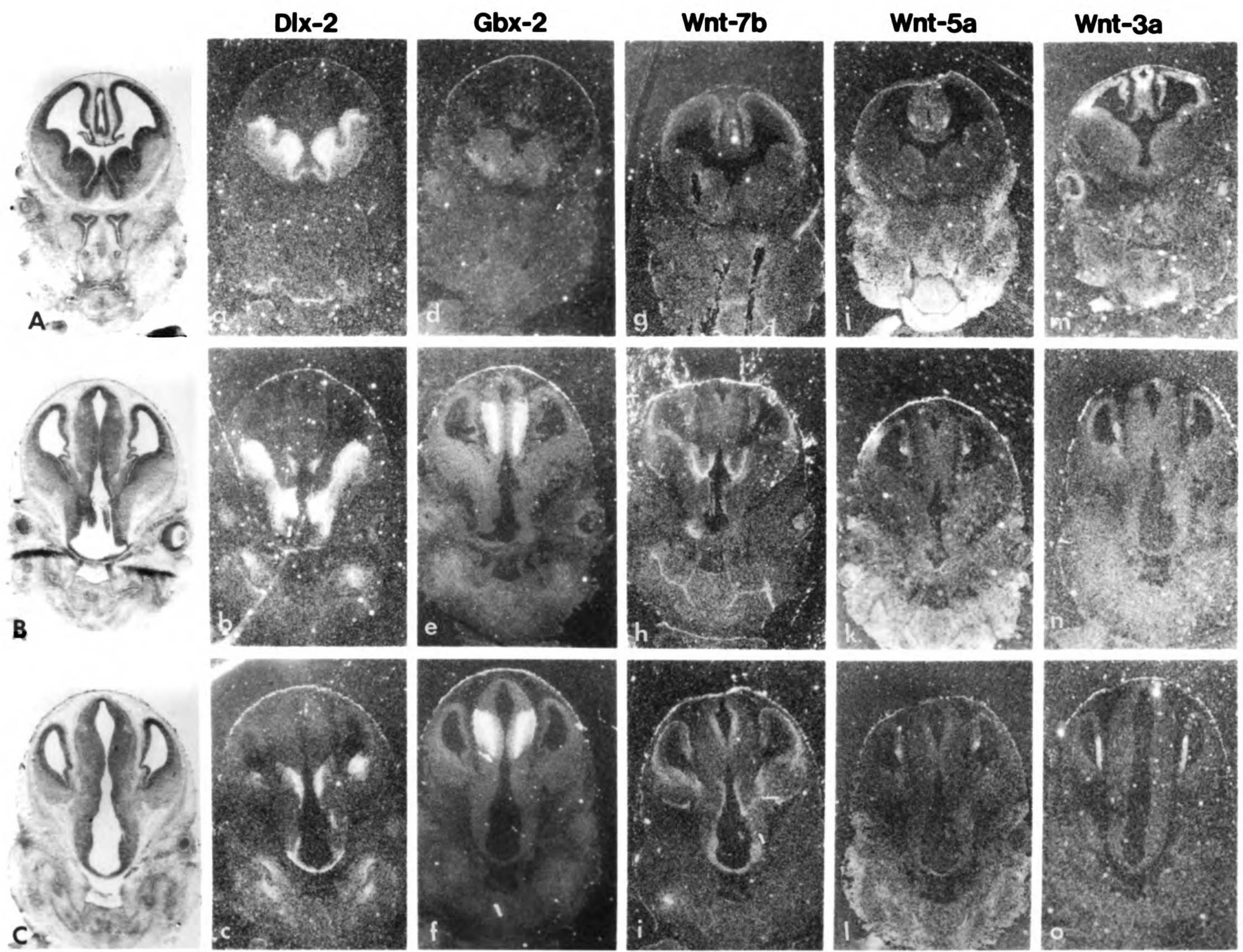


Figure 1



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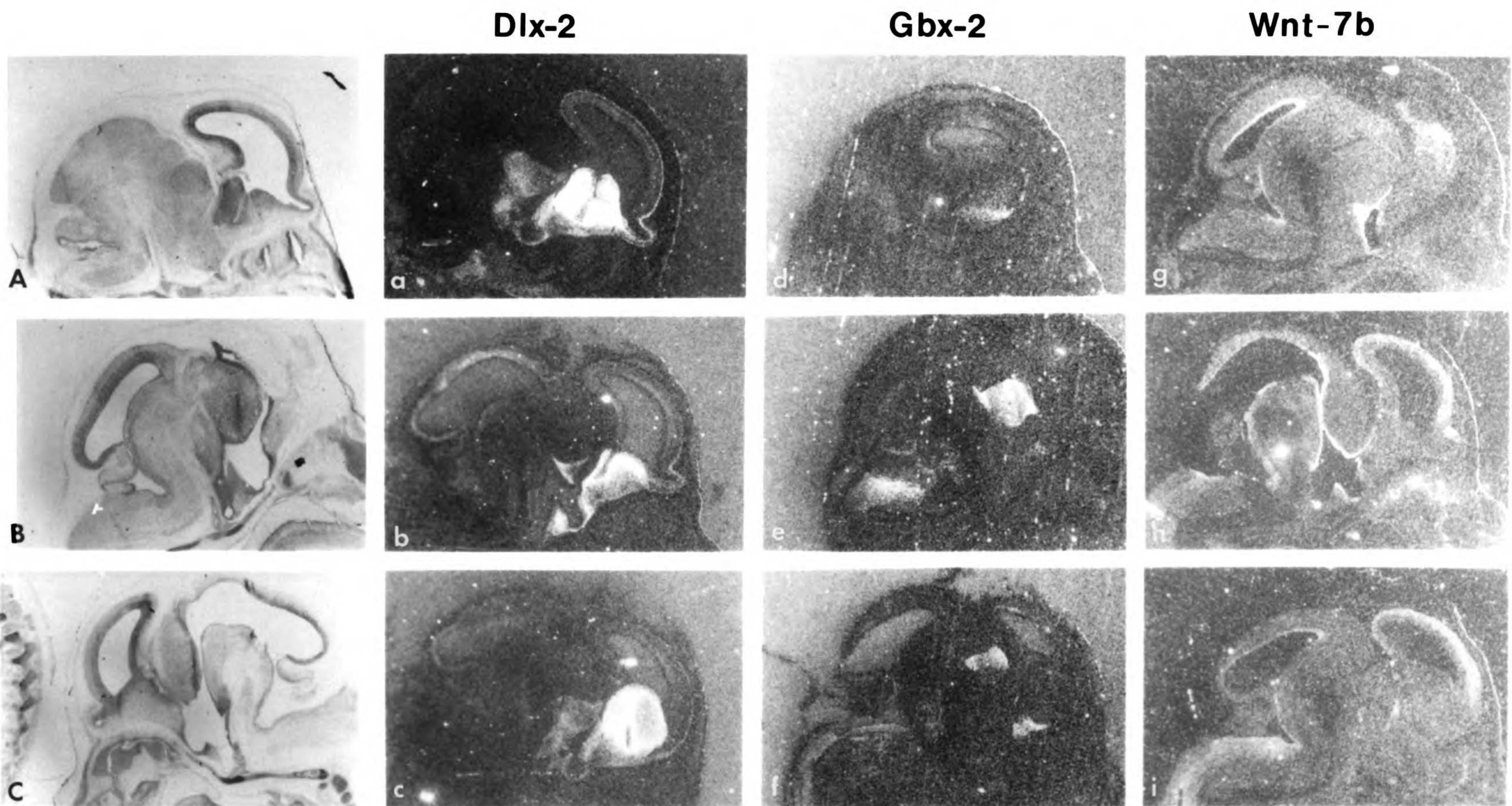
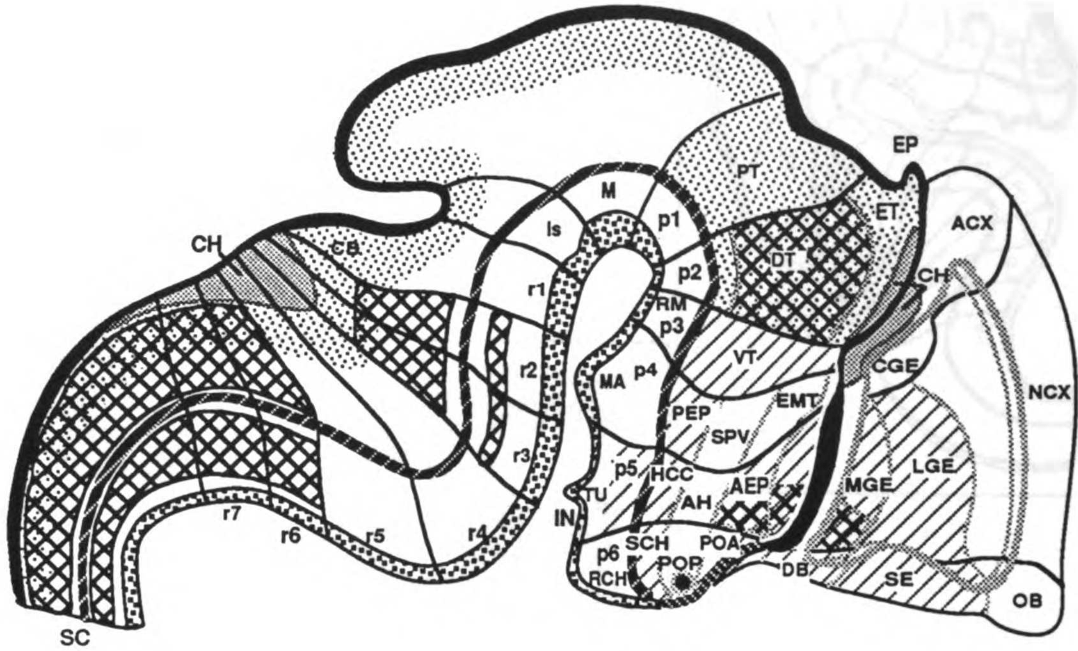
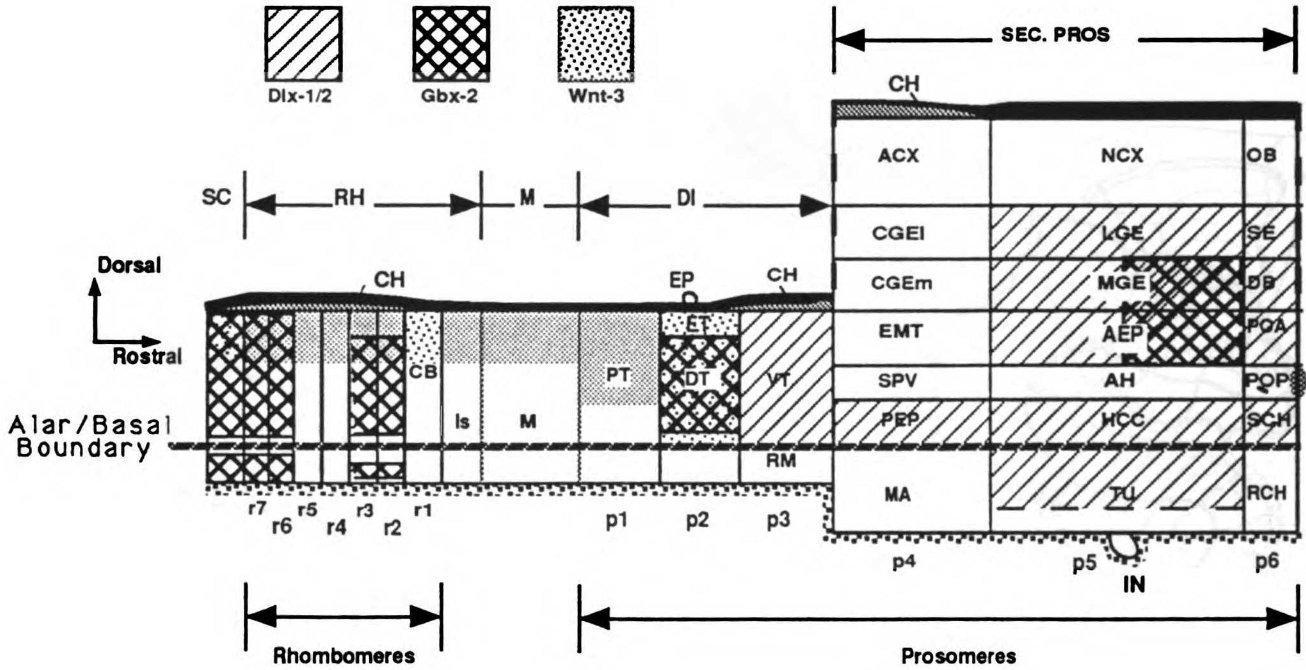


Figure 3

Figure 4





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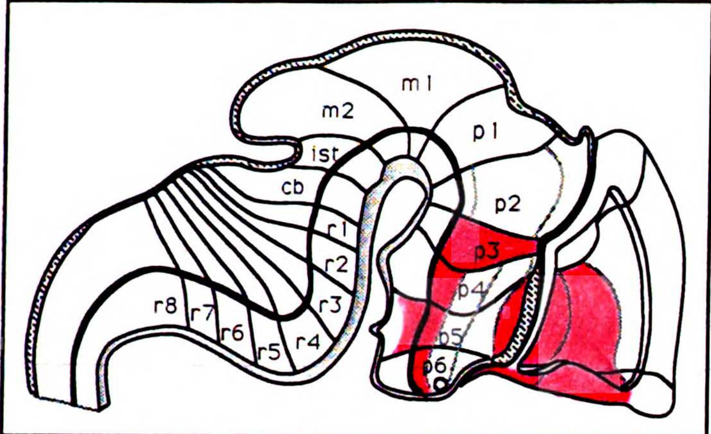

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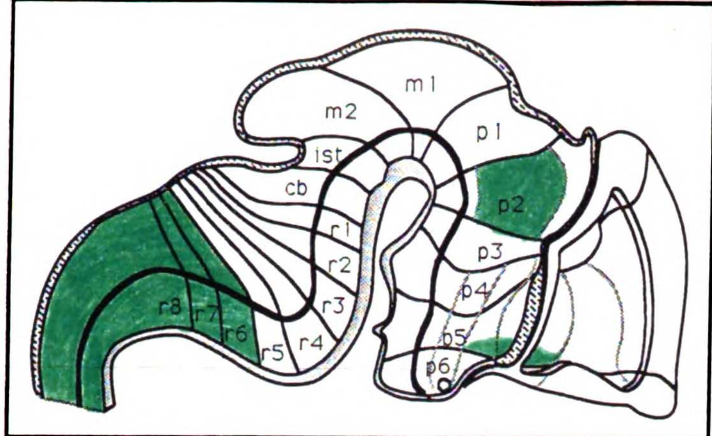

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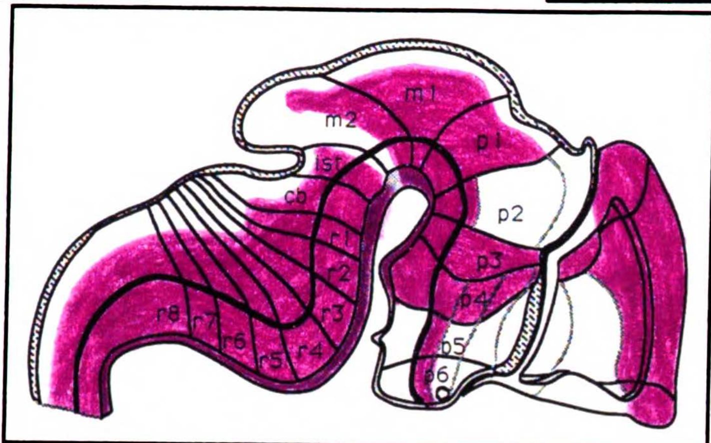

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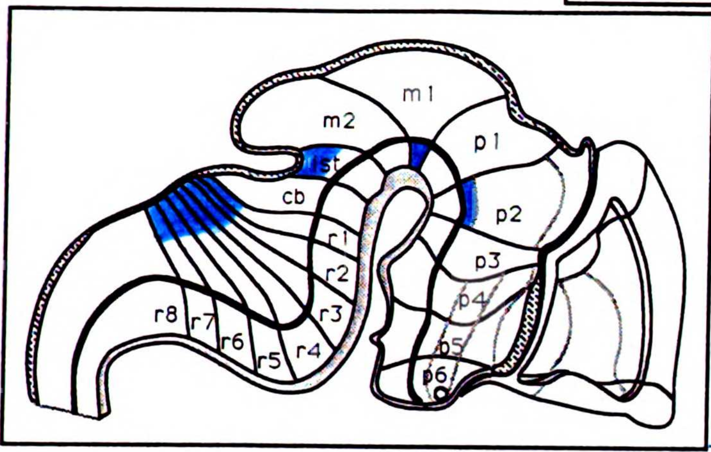

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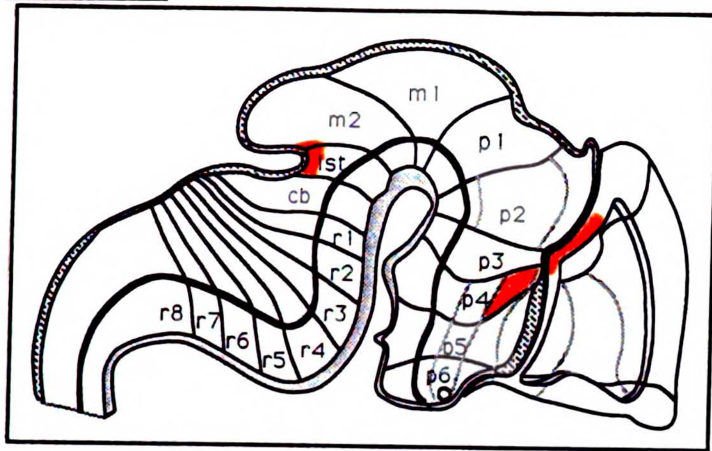
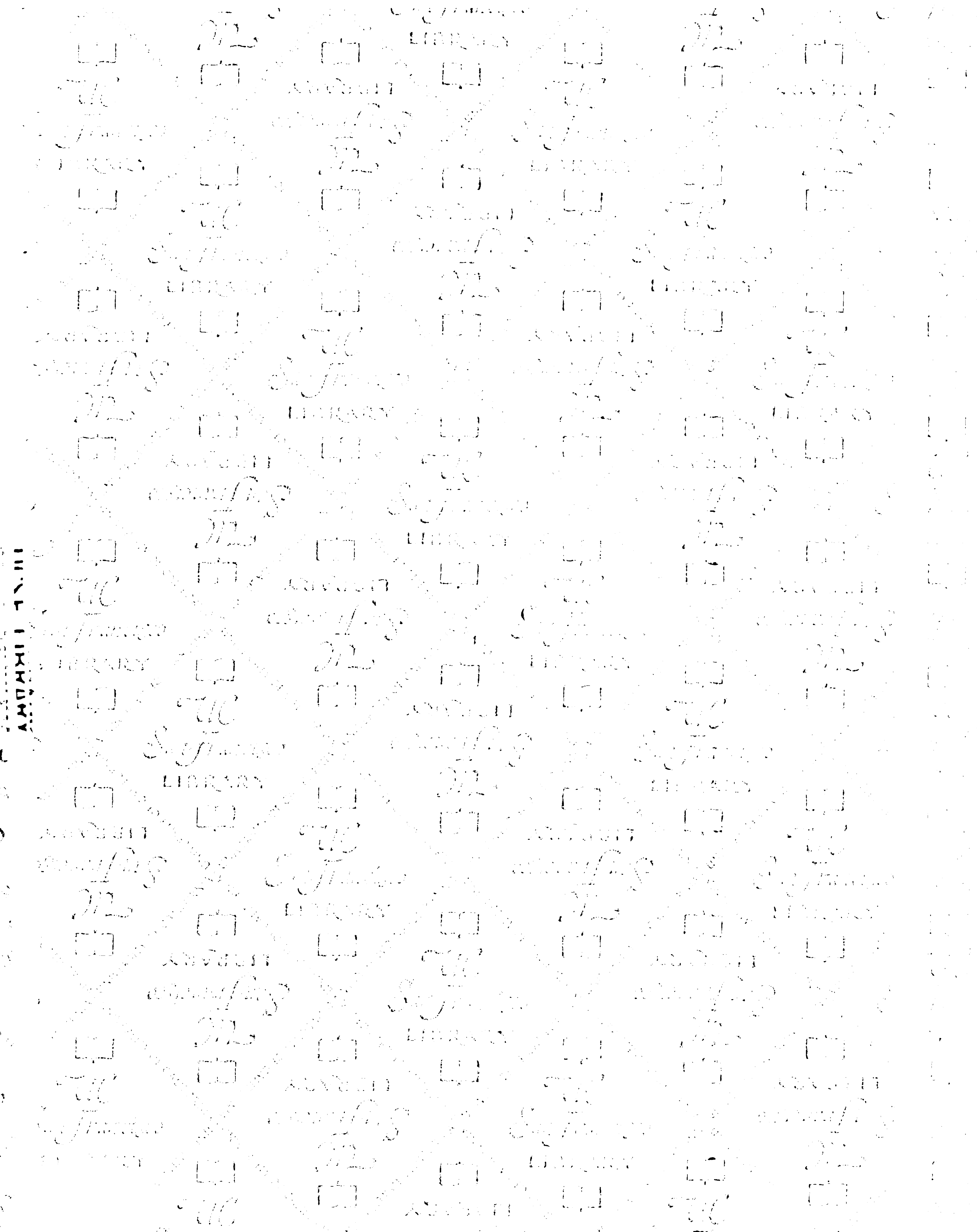


Wnt-3a

Figure 6



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