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Role of LIM-HD Genes in the Specification of Cell Identity

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Members of the LIM gene family perform crucial functions at multiple stages in the development of several systems. A subclass of the LIM gene family encodes a group of LIM-homeodomain (LIM-HD) transcription factors. The diversity of LIM gene function is partly due to complex regulatory interactions between LIM-HD, other LIM proteins and co-factors of LIM (CLIM) molecules. Here, we review studies on LIM-HD genes in *Drosophila* and in vertebrate systems that highlight the crucial roles this family plays in the specification of cell identity, and reveal important features of their mechanism of action. These findings motivate an examination of LIM-HD genes in the developing and mature cerebral cortex, where their roles are not well understood. Several LIM and Clim genes display distinct and dynamic expression patterns in the cortex throughout embryonic and postnatal stages. Close examination of these patterns suggests roles for some LIM genes that may further extend the current models of LIM gene function.

Key Words: Apterous, Spinal cord, Motoneuron, Interneuron, LIM code, Neuroepithelium, Cortical plate, Regional specification, Patterning, Cortical hem

Introduction

The development of an organism involves several levels of controlled interaction between and within cells, mediated by a vast array of different types of molecules. Some of these molecules act as key regulators of crucial steps in the development of specific structures. Here, we focus on a particular family of molecules encoded by LIM genes, which are emerging as regulators at multiple levels in the development of several organisms, displaying roles in tissue patterning, cell fate specification, and growth. The defining members of this family, *lin11* from *C. elegans*, *Isl1* from rat, and *mec3* from *C. elegans*, give the acronym "LIM."

The LIM gene family encodes molecules that contain two cysteine-rich LIM domains, which mediate protein-protein interactions. Some members contain an additional DNA binding homeodomain, and are called LIM-HD proteins, which function as transcription factors. For these proteins, DNA binding is thought to be modulated by the LIM domains, which interact with co-factors (Sanchez-Garcia et al. 1993, Agulnick et al. 1996). In such a model, several kinds of regulatory interactions are possible, giving rise to multiple and distinct roles for these genes. We will begin by reviewing studies that set up the model for LIM-HD gene function, focusing on the role of LIM-HD gene *apterous* in the *Drosophila* wing disc. Then, we will discuss work in the central nervous system (CNS) of invertebrates and vertebrates where LIM-HD genes specify neuronal identity in the respective developing nerve cords. In the vertebrate spinal cord, this involves combinatorial interactions that significantly extend the *apterous* model of LIM-HD gene function. These findings motivate a close examination of the role of this gene family in other parts of the CNS, such as the cerebral cortex, the most complex and least understood structure of the vertebrate CNS. We will conclude with a review of current knowledge about the expression and function of LIM genes in the embryonic and mature cerebral cortex.

Role of LIM-HD Gene *apterous* in *Drosophila* Wing Development

In *Drosophila*, the *apterous* (*ap*) gene encodes the most well studied protein of the LIM-HD family, other members of which include *Awh, dlim1*, *dlim3*, and *isl* (Cohen et al. 1992, Curtiss & Heilig 1997, Thor & Thomas 1997, Thor et al. 1999, Lilly et al. 1999). *ap*

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Table 1. *LIM-HD genes in different species and their roles in development*

Organism	LIM-HD Gene	Important Role in	Reference
Mouse	*Lhx1*	Head induction, axis formation, intermediate mesoderm differentiation	Shawlot & Behringer 1995 Shawlot et al. 1999, Tsang et al. 2000, Kinder et al. 2001
	Lhx2	Early cortical development, eye, erythrocyte development	Porter et al. 1997, Bulchand et al. 2001, Monuki et al. 2001
	Lhx3	Motoneuron and interneuron specification in spinal cord, pituitary gland organogenesis	Sheng et al. 1996, Sharma et al. 1998, Sharma et al. 2000
	Lhx4	Motoneuron specification, pituitary gland organogenesis	Sharma et al. 1998, Raetzman et al. 2002
	Lhx5	Hippocampal morphogenesis	Zhao et al. 1999
	Lhx6	Striatal interneuron specification	Marin et al. 2000
	Lhx7/8	Striatal interneuron specification, tooth development	Marin et al. 2000, Shibaguchi et al. 2003
	Lhx9	Gonad formation	Birk et al. 2000
	Lmx1a	Roof plate formation in the CNS, neuronal migration in the cortex	Millonig et al. 2000, Costa et al. 2001
	Lmx1b	Development of skull (calvarial) bones, anterior segment of the eye, and mesencephalic dopaminergic neurons, podocyte differentiation, motoneuron axon trajectory, dorsal limb fate specification, morphogenesis of isthmic organizer	Chen et al. 1998a, b Adams et al. 2000, Kania et al. 2000, Pressman et al. 2000, Smidt et al. 2000, Miner et al. 2002, Rohr et al. 2002
	Isl1	Motoneuron specification, pancreatic mesenchyme and islet cell formation	Pfaff et al. 1996, Ahlgren et al. 1997
	Isl2	Expressed in motoneurons	Tsuchida et al. 1994, Kania et al. 2000, Shirasaki & Pfaff 2002
Drosophila	*dlim1*	Leg and antennal development, neuronal subclass specification in the ventral nerve cord	Lilly et al. 1999, Tsuji et al. 2000
	dlim3	Motoneuron specification	Thor et al. 1999
	isl	Axon pathfinding and neurotransmitter identity	Thor & Thomas 1997
	ap	Wing, haltere, muscle and leg development, axon pathfinding and neurotransmitter identity, juvenile hormone synthesis, sexual functioning	Altaratz et al. 1991, Ringo et al. 1991, Bourgouin et al. 1992, Cohen et al. 1992, Ringo et al. 1992, Lundgren et al. 1995, Benveniste et al. 1998, Ghazi et al. 2000, Pueyo et al. 2000
	Awh	Establishment of abdominal histoblasts and salivary gland imaginal rings	Curtiss & Heilig 1995, 1997
C. elegans	*lin11*	Specification of olfactory and chemosensory neurons, vulval patterning, uterine morphogenesis, function of AIZ thermoregulatory interneurons	Hobert et al. 1998, Newman et al. 1999, Sarafi-Reinach et al. 2001, Gupta et al. 2003
	mec3	Touch receptor neuron differentiation	Duggan et al. 1998, Way & Chalfie 1988
	lim4	Specification of AWB olfactory neuron fate	Sagasti et al. 1999
	ttx3	Regulation of AIY interneuron differentiation and function	Hobert et al. 1997, Altun-Gultekin et al. 2001
	lim6	Axonal morphology and function of a subset of GABAergic motoneurons, chemosensory representation, maintenance of ventral nerve cord architecture	Hobert et al. 1999, Pierce-Shimomura et al. 2001, Aurelio et al. 2003
	ceh-14	Thermosensory function in AFD neurons, maintenance of ventral nerve cord architecture	Cassata et al. 2000, Aurelio et al. 2003

has been studied extensively in the context of wing development where it plays the role of a dorsal selector gene. During *Drosophila* embryogenesis, groups of cells called imaginal discs are set aside, which proliferate during larval development, and eventually differentiate into particular adult structures such as antenna, wing and leg. *ap* is expressed in the wing imaginal discs starting from second instar larval stage. Its expression is localized to regions that give rise to dorsal compartment of the wing blade and the notum (figure 1; Cohen et al. 1992, Blair 1993). The role of *ap* in wing development is evident in different *ap* mutants where the severity of wing phenotype varies from mild defects in the hinge region of the wing to a complete lack of wings (Stevens & Bryant 1985, Cohen et al. 1992).

In the presumptive dorsal wing, hinge, and notum region of the wing imaginal disc, the *vein* (*vn*) ligand activates EGFR signaling and establishes the expression of *ap* (Wang et al. 2000). This is stably inherited by the dorsal cells of the imaginal disc, which move away from the region of high EGFR signaling as proliferation proceeds, and eventually form part of the wing structure. In contrast, cells in regions of continued high EGFR signaling become the notum. The migration of Ap expressing cells away from the EGFR signaling zone creates a dorsal compartment of cells that express Ap outside the region of EGFR signaling, juxtaposed to ventral cells that do not express *ap* (Zecca & Struhl 2002a,b). The boundary between these two cell types becomes the dorsoventral (D/V) boundary of the wing (figure 1). Cells from the two compartments do not normally mix. However, experiments where clones of dorsal cells were made *ap* deficient demonstrated that these cells could now violate the boundary restriction and migrate over to the ventral side and start expressing ventral genes.Likewise, ventral cells that ectopically expressed *ap* were also able to cross the compartment boundary and express dorsal-specific genes (Diaz-Benjumea & Cohen 1993, Blair et al. 1994). This suggested that segregation of cells into dorsal and ventral types is *ap* dependent. Indeed, two dorsal specific transmembrane proteins regulated by Ap, Capricious and Tartan, initially establish this affinity difference between the dorsal and ventral cells (Milan et al. 2001a).

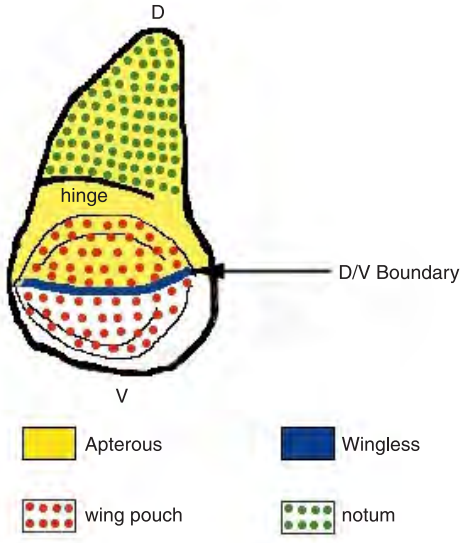


Figure 1. Apterous expression during wing disc development in *Drosophila*. Ap expression (yellow) is limited to the dorsal portion of the presumptive wing blade (red dots). Ap is also expressed in the presumptive notum (green dots), and in the hinge. The dorsoventral boundary of the presumptive wing blade expresses Wg (blue).

Dorsal cells express *fringe* (*fng*), one of the downstream target genes of Ap, which is essential for maintaining the D/V boundary restriction in the second instar stage (Irvine & Wieschaus 1994). At this stage, in these cells, Ap also induces the expression of the Notch ligand, Serrate (Ser) and suppresses the expression of another ligand Delta (Dl). Ventral cells close to the D/V boundary express Dl in late second instar larval stage (Kim et al. 1995, de Celis & Bray 1997, Milan & Cohen 2000). Thus cells in the dorsal and ventral compartments express different Notch ligands. Cells at the boundary of each compartment interact with each other and become specialized to express "boundary-specific" genes such as *wingless* (*wg*). Fng mediates this specialization by causing dorsal cells to be refractory to Ser, blocking Ser-Notch signaling within the dorsal compartment. At the same time, Ser-expressing dorsal cells activate Notch signaling in ventral cells that are immediately adjacent to them (Panin et al. 1997, Fleming et al. 1997, Klein & Arias 1998). Fng expression in dorsal cells also makes them responsive to Dl, allowing Notch activation in dorsal cells that are immediately adjacent to Dl-expressing ventral cells. This causes symmetric activation of Notch on either side of the D/V boundary, which in turn activates margin-specific genes like *wg*, *cut*, and *vestigial* in the cells at the interface between the two compartments (Diaz-Benjumea & Cohen 1995). Thus a Wg secreting

organizer is established at the D/V boundary, and this center is essential for wing margin formation and further D/V organization (Ng et al. 1996).

Loss of *fng* in dorsal cells or ectopic Fng expression in ventral cells causes them to violate the D/V boundary, due to a disruption of the affinity differences between the two compartments (Rauskolb et al. 1999, O'Keefe & Thomas 2001). This effect is not as strong as seen in *apterous* mutant cells, however, suggesting that Ap can regulate affinity differences via a pathway independent of Fng (Milan & Cohen 2003). Also, Fng cannot confer dorsal identity to the dorsal cells in the absence of Ap (O'Keefe & Thomas 2001). This function of Ap is carried out by another downstream molecule, the muscle-specific homeobox (Msh) protein (Milan et al. 2001b).

Mechanism of Action of LIM-HD Protein Ap

The first indications of how LIM-HD proteins might function came from *Xenopus* and mammalian studies. Biochemical approaches yielded a co-factor XLdb1 (also called NLI/Chip/Clim in other systems), which was found to bind to LIM-HD gene *Xlim1* and synergize with aspects of its function (Agulnik et al. 1996). The binding was found to occur at sites separate from the DNA binding site on the LIM-HD molecule, and also independent of the dimerization domain on the Ldb1 protein (Breen et al. 1998). In mammalian systems, the homologous molecule (called NLI) was found to bind specific LIM-HD proteins, self-dimerize, and the dimeric protein shown to participate in forming homo- and heteromeric complexes with single or different LIM-HD molecules (Jurata et al. 1996, Jurata & Gill 1997, Bach et al. 1997, Jurata et al. 1998).

These findings were paralleled in the *Drosophila* wing, where genetic evidence supported a tetrameric model of LIM-HD and co-factor molecules (figure 2). Chip, a widely expressed protein in *Drosophila*, acts as a cofactor of Ap, and this interaction is required for the transcriptional activity of Ap (Morcillo et al. 1997). Relative amounts of Chip and Ap are important for D/V compartmentalization of the wing (Fernandez-Funez et. al. 1998). Chip has a self-dimerization domain (DD) as well as a LIM-interacting domain enabling two molecules each of Ap and Chip to form a tetrameric complex. (figure 2a) The LIM domain of Ap interacts with Chip while the homeodomain functions to bind DNA. This tetrameric complex of 2(Chip): 2(Ap) mediates the transcriptional activity of Ap and can activate downstream target genes (Fernandez-Funez et al. 1998, Milan & Cohen 1999, van Meyel et al. 1999, Rincon Limas et al. 2000). Formation of a 2(Chip): 2(Ap): DNA complex stabilizes the Ap protein *in vivo* (Weihe et al. 2001). In order to analyze the nature of the interaction between Ap and Chip, chimeric constructs were used. A chimeric protein having only the DD of Chip and homeodomain of Ap can replace Ap *in vivo*: it can rescue the wing phenotype in Ap or Chip mutants as well as in Ap-Chip double mutants (figure 2b, Milan & Cohen 1999, van Meyel et al. 1999). Presumably the chimeric protein dimerizes via the DD, forming a dimer that is capable of transcriptional activity (figure 2b) Since this chimeric construct is sufficient to substitute for both Chip and Ap function and produce a normal wing, this suggests that one of the major roles of Chip in this system is to dimerize Ap (van Meyel et al. 1999, Milan & Cohen 1999).

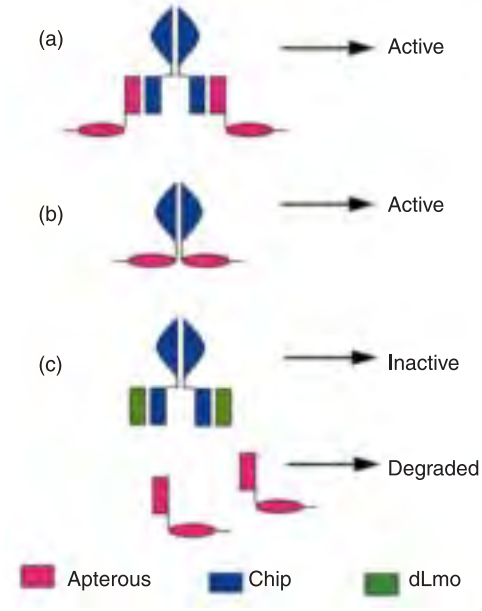


Figure 2. Tetrameric model for LIM-HD activity. Two molecules of Ap (red) bind to a Chip dimer (blue), forming a transcriptionally active tetrameric complex **(a)** A hybrid protein containing the HD of Ap (red) fused to the dimerization domain of Chip (blue) also forms a functional complex; **(b)** dLmo molecules (yellow) compete with Ap for binding to Chip, forming an inactive dLmo-Chip complex, while free Ap molecules are degraded **(c).**

A further complexity to the system is added due to the presence of a "LIM-only" protein dLmo, encoded by the *beadex* gene, which serves to regulate the amount of functional tetramer produced. This protein has two tandem LIM domains but lacks the DNA-binding homeodomain (figure 2c, Zhu et al. 1995). Since the interaction of Chip and Ap occurs via the LIM domain, dLmo can compete with Ap for the cofactor Chip, compromising Ap stability (figure 2c, Milan et al. 1998, Milan & Cohen 1999, Weihe et al. 2001). However, *dlmo* is also a target gene of Ap in the dorsal compartment in the second instar stage; therefore there is a time window of Ap activity during which dLmo accumulates and can then compete for Chip. In this fashion, Ap controls its own activity temporally via a feedback mechanism. This temporal control is required for the proper development of the wing (Milan & Cohen 2000).

LIM-HD Proteins in *Drosophila* Neural Development

Similar to the wing, cells in neuronal structures need to be specified according to positional identities such as "dorsal" and "ventral." They also need to develop particular features specific to neurons such as the correct neurotransmitter identity and an appropriate pattern of innervation. "Cell fate specification" in neurons can therefore be assessed with respect to these features. LIM-HD genes function in the specification of both these aspects in neurons.

During embryonic development in *Drosophila*, Ap is expressed in a subset of interneurons in the ventral nerve cord, where it is required for proper axonal pathfinding and fasciculation of neurons (Lundgren et al. 1995, Herzig et al. 2001). Ap is expressed in three interneurons per *Drosophila* embryonic ventral nerve cord (VNC) hemisegment. In addition, each thoracic hemisegment has a lateral cluster of 4 neurons that express Ap (Lundgren et al. 1995). All the neurons, except the Tv neuroendocrine neuron of the lateral cluster, join to form an Ap fascicle within the VNC (figure 3; Lundgren et al. 1995). Ap is required for proper axonal pathfinding by all the neurons in the apterous fascicle (Lundgren et al. 1995). In contrast, Ap is not required for pathfinding in the Tv neurons, but is required for their neurotransmitter identity, namely, FMRFamide expression, which is a characteristic for these neurons (Schneider et al. 1991, Benveniste et al. 1998). More recently it has been shown that Ap cooperates with a zinc finger protein, Squeeze and with a retrograde BMP signal from the Tv neuron target site to initiate the FMRFamide expression in these cells (Allan et al. 2003).

The axonal pathfinding by most Ap expressing neurons is dependent on formation of the 2(Chip): 2(Ap) tetrameric complexes that are also necessary for wing development (van Meyel et al. 2000). When the LIM domains of Ap, which are required for interaction with Chip, are interchanged with those of other members of the family, a normal wing is produced. However, axonal pathfinding becomes defective, indicating that different LIM domains are responsible for different functions. Hence only a selective rescue of *ap* phenotypes is observed when LIM domains are interchanged between related

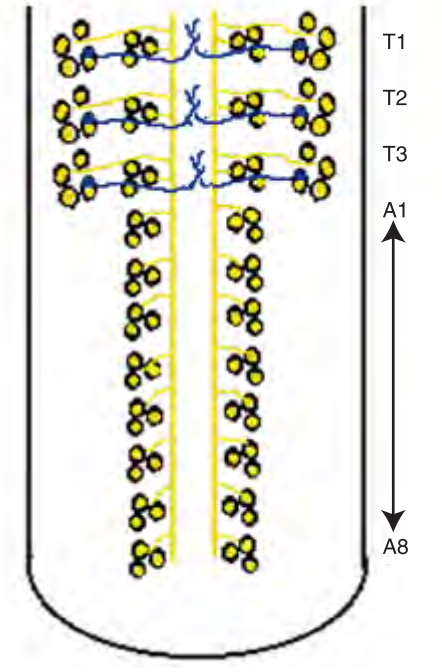


Figure 3. Apterous expressing neurons in the *Drosophila* VNC. At each segmental level, clusters of 3 neurons send out axons forming an Apterous fascicle (yellow). At thoracic levels (T), an additional cluster of 4 neurons expresses Ap, from which 3 join the Ap fascicle. The Tv neuron, which also expresses FMRFamide (blue), instead projects in a different path. Ap has distinct functions in the Ap-fascicle neurons and the FMRFamide expressing neurons. Reprinted from Cell, Vol 113, pg 73-86. Allan et al. Specification of neuropeptide identity by the integration of retrograde BMP signaling and a combinatorial transcription factor code. Copyright (2003), with permission from Elsevier.

LIM-HD genes (O'Keefe et al. 1998). In this case, the observation that different LIM domains are able to perform different functions suggests that the mere formation of a tetramer is not sufficient for activation of all downstream genes. There may be other interactors that distinguish between different LIM domains and can regulate the ability of the tetramer to activate specific downstream genes (O'Keefe et al. 1998). This also indicates that the different Chip dependent activities of Ap in distinct tissues are context specific and probably require cell type specific interactions.

Drosophila islet (*isl*), another LIM-HD gene, is expressed in subsets of motoneurons and interneurons, including the dopaminergic and serotonergic interneurons, in the VNC. *isl* loss of function, like *ap* loss of function, does not affect the generation and survival of these neurons but affects their pathfinding and neurotransmitter identity (Thor & Thomas 1997). Lim3, another *Drosophila* LIM-HD protein, is also expressed in a subset of motoneurons and interneurons in the VNC, and mutants of *lim3* exhibit pathfinding defects in these neurons (Thor et al. 1999). Specifically, some motoneurons that express *lim3* also express *isl*, and project in the transverse nerve and in the ISNb branch of the intersegmental nerve to muscles of the embryonic abdominal hemisegment. A different set of motoneurons that express only *isl* project in the ISNd branch of the intersegmental nerve to a different set of muscles (figure 4a). In *lim3* mutants, ISNb neurons project ectopically to ISNd target sites. Conversely, when *lim3* is misexpressed in ISNd neurons, they project incorrectly to the ISNb target sites (figure 4b). This indicates that a "LIM-code" for motor neuron pathfinding exists in the *Drosophila* VNC (Thor et al. 1999). This scenario is paralleled and expanded in the vertebrate spinal cord, where combinatorial expression of LIM genes encode the cell identity of distinct classes of neurons.

A LIM Code in the Vertebrate Spinal Cord

In the vertebrate spinal cord, a tube-like structure that is divided into dorsal and ventral halves, specific populations of neurons occupy discrete positions along the dorsoventral axis. Each group has distinct patterns of innervation and therefore subserves different functions. All neurons are generated from proliferating precursors in the

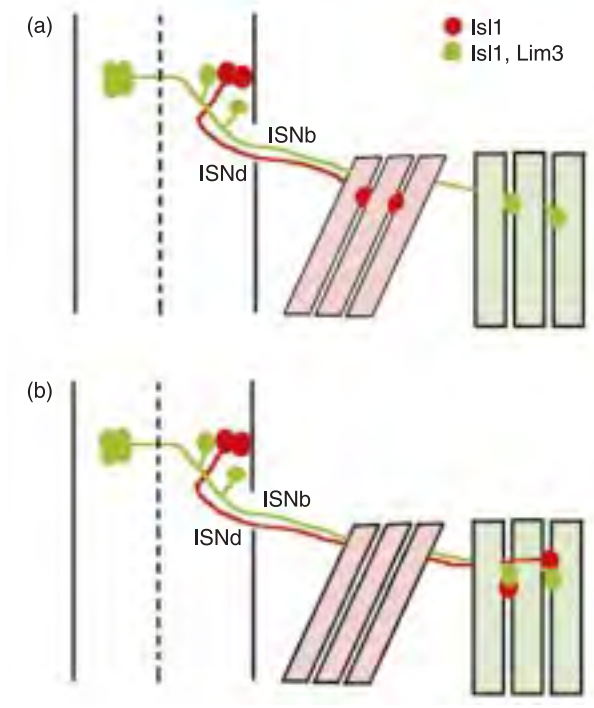


Figure 4. A "LIM code" controls motoneuron pathfinding in the *Drosophila* VNC. Normally, neurons that express only Isl (red) project via the ISNd to a particular group of muscles. Neurons expressing both Isl and Lim3 (green) project via the ISNb to a different group of muscles (a). When Lim3 is misexpressed in the ISNd group of neurons, they project incorrectly to the ISNb target muscles; (b). Reprinted from ***Nature, Vol 397.*** Thor et al. A LIM-homeodomain combinatorial code for motor-neuron pathway selection. Copyright (1999), with permission from Nature Publishing Group. (site : http://www.nature.com/)

ventricular zone, and migrate outward after becoming postmitotic, to occupy their final positions, and send out axons to the appropriate targets (reviewed in Tanabe & Jessell 1996, Shirasaki & Pfaff 2002).

During early development, secreted molecules from signaling centers at the dorsal and ventral midline initiate the process of patterning in the spinal cord. In the dorsal spinal cord, the roof plate secretes Bone morphogenetic proteins (BMPs), which are essential for specification of certain dorsal interneurons (Liem et al. 1995, 1997). The ventral spinal cord is patterned by Sonic Hedgehog (Shh), a signaling molecule that is secreted by the notochord as well as the floor plate in the spinal cord (Echelard et al. 1993, Marti et al. 1995). Shh is essential for generation of motoneurons and certain ventral interneurons (Roelink et al. 1995, Marti et al. 1995, Ericson

et al. 1995, 1996, Wijgerde et al. 2002, Shirasaki & Pfaff 2002). Both the BMPs and Shh are morphogens, acting in a concentration dependent manner. In the ventral spinal cord, specification of diverse cell types occurs because different concentrations of Shh results in differential activation of transcription factors of the bHLH family as well as members of the Pax, Dbx, Nkx, and Irx families of homeobox genes in the proliferating progenitors (Burrill et al. 1997, Ericson et al. 1997, Osumi et al. 1997, Pierani et al. 2001, Briscoe et al. 1999, 2000). The combinatorial expression of these transcription factors creates specific types of progenitor populations, which will form the V0-V3 interneurons, and the motoneurons (Briscoe et al. 2000).

A "LIM code" i.e. a combinatorial expression of particular LIM genes, specifies cell identity and acts at multiple stages of motoneuron specification. An *early* LIM code differentiates a particular motoneuron subclass from an adjacent interneuron population (Sharma et al. 1998). After this stage, specific subtypes of motoneurons are created by a different, *later* acting LIM code, which was the first combinatorial code proposed for the members of this gene family (Tsuchida et al. 1994, Kania et al. 2000, Sharma et al. 2000). In contrast, in the dorsal spinal cord, although LIM genes are expressed, there is as yet no evidence of a "LIM code" to specify distinct dorsal interneuron subtypes (Helms et al. 2003).

An Early LIM code for Motoneuron Versus Interneuron Specification

Motoneurons and V2 interneurons arise from adjacent domains in the spinal cord (Lee & Pfaff 2001). In mouse, cells that will become motoneurons express the LIM-HD gene Isl1 in the final division of their cell cycle, just before they become postmitotic (Ericson et al. 1992). One sub-class of motoneurons, those whose axons exit ventrally from the neural tube (vMNs), also *transiently* express Lhx3 and a related gene Lhx4 (Sharma et al. 1998). Interneurons never express Isl1, but the V2 class of interneurons expresses Lhx3 and Lhx4 before they become postmitotic (Sharma et al. 1998). Thus an "early" LIM code for V2 interneuron versus vMN specification was hypothesized, in which the presence of Isl1 would direct motoneuron fate, and the additional presence of Lhx3/4 would lead to a vMN fate. In the absence of Isl1, however, an interneuron fate would be expected, and the presence of Lhx3/4 in this situation would lead to a V2 interneuron fate (Tanabe et al. 1998, Thaler et al. 2002).

Gene Perturbation Studies Support the LIM Code Hypothesis

Studies using knockout mice demonstrate that the LIM code does indeed control cell fate in these neurons. *Isl1* knockout mice have no motoneurons, indicating an absolute requirement for Isl1 in development of the entire motoneuron pool (Pfaff et al. 1996). Mice deficient in *either* Lhx3 or Lhx4 show no defect in motoneuron or interneuron development, suggesting redundancy in function of these two related genes (Sharma et al. 1998). However, the double knockout for Lhx3 and Lhx4 reveals a complete loss of V2 interneurons, confirming that at least one of Lhx3 or Lhx4 is required for V2 interneurons to develop. In addition, there is a defect in the vMNs, such that they now exit dorsally (Sharma et al. 1998).

Other studies in the chick spinal cord show that ectopic expression of *Lhx3* in the *dorsal* (non-*Isl1* expressing) spinal cord triggers V2 interneuron generation, indicating that Lhx3 is sufficient to drive the specification of at least one type of ventral interneuron (Tanabe et al. 1998, Thaler et al. 2002). Furthermore, ectopic expression of *Lhx3* together with *Isl1* is sufficient to produce vMNs in the dorsal spinal cord, where they would ordinarily never occur (Tanabe et al. 1998). Thus the differential expression of *Isl1* and *Lhx3* genes in vMNs and V2 interneurons serves as a "code" that directs the specification of these different neuronal types.

How the LIM Code Works

At the molecular level, though, this "LIM code" presents a challenging problem. According to the functional model for LIM genes based on studies in *Drosophila*, a functional tetramer consisting of two LIM-HD molecules, bridged by 2 Clim molecules, is required for downstream activity (van Meyel et al. 1999, Rincon-Limas et al. 2000). The cofactor Clim2 (also termed NLI, Ldb1) is present in all spinal cord progenitors, hence the formation of a tetramer comprising 2(Clim2): 2(Lhx3) would be predicted in V2 interneurons

(Thaler et al. 2002). However, this particular tetramer could also form in the motoneuron progenitors that will become vMNs, since these cells express *Lhx3* and *Clim2*. These neurons are presumably prevented from becoming V2 interneurons due to the presence of Isl1, which is present in all motoneurons. The crucial issue then is how this can be achieved at a molecular level.

Elegant experiments by Thaler et al. (2002) resolved the problem of V2 interneuron versus vMN specification (reviewed in Sockanathan 2003). They electroporated the *dorsal* spinal cord of chick embryos *in ovo*, with different constructs, and assayed for the generation of V2 interneurons and vMNs. As expected, Thaler et al. (2002) show that a tetrameric complex of 2(Clim2): 2(Lhx3) generates V2 interneurons(figure 5). In an attempt to characterize the functionally critical regions of this tetrameric complex, they electroporated *defective* constructs. A truncated *Lhx3* construct lacking the LIM domain, or containing a defective, non-DNA-binding homeodomain, fails to generate V2 interneurons. However, V2 interneurons are produced by electroporation of a fusion construct containing the dimerization domain (DD) of Clim2 and homeodomain (HD) of Lhx3 (Clim2DD-Lhx3HD). As in the *Apterous* model, these experiments identify the DD of Clim2 and the HD of Lhx3 as the essential domains for a functional complex.

In vMNs, however, three different types of tetramers could potentially form: 2(Clim2): 2(Lhx3); 2(Clim2): 2(Isl1) and possibly a hetromeric complex of 2(Clim2): 1(Lhx3): 1(Isl1). If any of these tetramers, alone or in combination, is adequate to produce motoneurons, then a (Clim2DD-Lhx3HD) fusion construct electroporated together with a (Clim2DD-Isl1HD) fusion construct should also produce vMNs. But this does not appear to be the case, however (Thaler et al. 2002). Further experiments using deletion constructs suggest that perhaps a higher order complex was being formed between Clim2, Isl1 and Lhx3. In vitro analysis showed that indeed, *hexameric* complexes of Clim2, Isl1 and Lhx3 are able to form, with Isl1 acting as the bridge between the two other proteins (figure 5). Further, using chimeric constructs, Thaler et al. (2002) show that the key domains of this complex are the Clim2DD, Isl1HD, and Lhx3HD (figure 5). Using protein binding studies, they also report that Isl1 is able to displace Lhx3 from Lhx3:Clim2 complexes. Additionally, Isl1 has a high affinity binding site for Lhx3, which is likely to sequester it and prevent the formation of Lhx3:Clim2 complexes that would drive V2 IN formation. Thus Thaler et al. (2002) showed that a *hexameric* complex of 2(Clim2):2(Isl1):2(Lhx3) is the functional complex required for vMN specification. These findings greatly extend the *Apterous* model of LIM gene action.

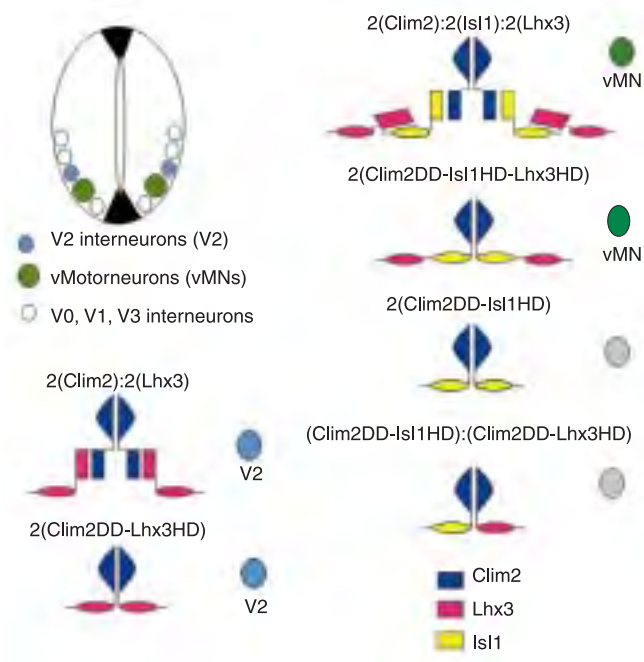


Figure 5. Molecular basis of the early LIM code in the vertebrate spinal cord: Hexameric model for LIM-HD function. A transcriptionally active tetrameric complex consisting of Lhx3 (red) and Clim2 (blue) molecules produces V2 interneurons. This activity is mimicked by a dimer of hybrid molecules containing the HD of Lhx3 (red) fused to the dimerization domain of Clim2 (blue). A hexameric complex consisting of Clim2 (blue), Isl1 (yellow), and Lhx3 (red) molecules gives rise to vMNs. This activity is mimicked by a dimer of a three-component hybrid, containing the dimerization domain of Clim2 (blue), and the HD of Isl1 (yellow) and the HD of Lhx3 (red). In contrast, a hybrid molecule containing the dimerization domain of Clim2 (blue) and the HD of Isl1 (yellow), does not give rise to either V2 interneurons or vMNs, suggesting that Isl1 activity by itself is inadequate for the specification of either identity. A heteromer consisting of Clim2 dimerization domains (blue) fused to the HD of Isl1(yellow) in one molecule and the HD of Lhx3 (red) in the other, is also inadequate in specifying vMN identity, supporting the hypothesis that a higher order complex forms when Clim2 is present together with Lhx3 and Isl1. Modified and reprinted from ***Trends in Neurosciences,*** Vol **26:2**, Sockanathan, S., Towards cracking the code: LIM protein complexes in the spinal cord, 57-59, Copyright (2003), with permission from Elsevier.

A LIM Code for Subtype Identity of Postmitotic Motoneurons

The idea that a "LIM code" could be involved in *motoneuron subtype specification* was first envisioned by Tsuchida et al. (1994) much before it was known that an early "LIM code" existed for specification of the entire motoneuron pool. In the chick spinal cord, motoneurons are organized into medial motor column (MMC), the lateral motor column (LMC) and the preganglionic motor column (PMC). The expression patterns of 4 LIM genes, Isl1, Isl2, Lhx1 and Lhx3 change rapidly in motoneurons soon after they become postmitotic (the chick ortholog of mouse Lhx4 has not been identified so far). All motoneurons express *Isl1* in the final division of their cell cycle (Ericson et al. 1992). As they start migrating outward from the ventricular zone, they start expressing *Isl2*. The LMC neurons become subdivided into lateral and medial groups (LMCl and LMCm, respectively). They are distinguished by upregulation of *Lhx1* expression and downregulation of *Isl1* selectively in the LMCl group. The MMC neurons are similarly divided into medial and lateral groups, MMCm and MMCl respectively. The MMCm group expresses *Lhx3* in maturity, while the MMCl does not. Meanwhile the PMC neurons begin to downregulate *Isl2* (figure 6).

Another distinguishing feature of motoneuron subtypes is the path their axons take when exiting the neural tube. Thus there are ventrally exiting and dorsally exiting motoneurons; the MMCm neurons fall into the vMN category. Interestingly, while all vMNs transiently express *Lhx3* in the *early* phase of their differentiation, only the MMCm subtype maintains continued expression of *Lhx3* at postmitotic stages.

Thus all postmitotic motoneurons except LMCl subtype express *Isl1*. On the other hand, *Isl2* is expressed in all except the PMC subtype. *Lhx3* is expressed in selectively in MMCm and *Lhx1* in LMCl. This combination of LIM gene expression is already established *before* mature neurons have segregated into columns and *before* axons have been extended to appropriate targets. The timing of this expression is therefore suggestive of a role in the subsequent events of motoneuron maturation. Further, these expression patterns suggested that a single LIM gene is not responsible for specifying a particular motoneuron subtype; rather, a distinct combination of LIM genes was essential for each subtype identity.

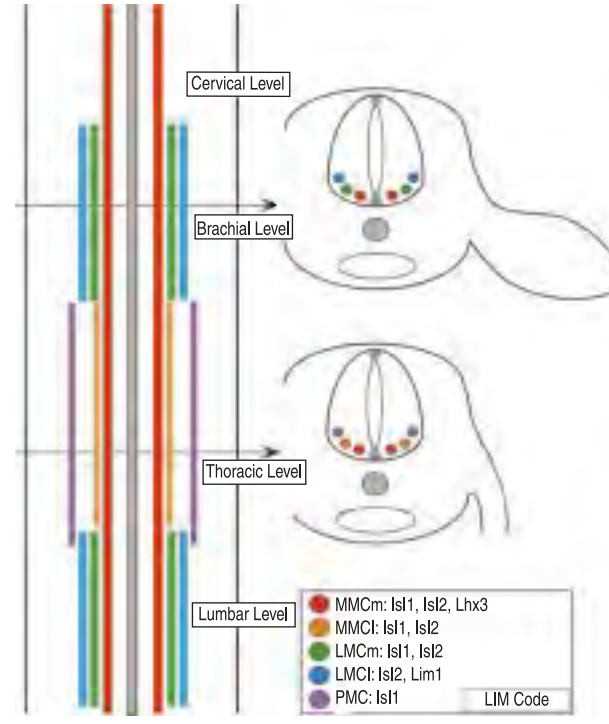


Figure 6. A 'LIM code' defines Motoneuron subtypes in the vertebrate spinal cord. Going from medial to lateral, the medial motor column has two components, medial and lateral (MMCm and MMCl, respectively). The MMCm runs along the entire rostrocaudal length of the spinal cord. At the thoracic level, the MMCl lies beside the MMCm. At branchial and lumbar levels, but not cervical and thoracic levels, the lateral motor column is present, which is also divided into medial and lateral components (LMCm and LMCl, respectively). An additional column, the preganglionic motor column (PMC), is present at the thoracic level. A LIM code distinguishes between the neuronal subtypes that are present in these columns. For simplicity, interneurons are not depicted in the figure. Modified, with permission, from Shirasaki & Pfaff 2002: ***The Annual Review of Neuroscience,*** Volume **25** ©2002 by Annual Reviews (www.annualreviews.org)

Gene perturbation studies in mice show that stable expression of *Lhx3* in all motoneurons converts all of these cells to the MMCm identity. This supports the idea that while all vMNs express *Lhx3* transiently in vivo, the continued presence of Lhx3 drives these neurons to a MMCm identity (Sharma et al. 2000). These findings fit well with the "LIM code" for MMCm neurons, since *Isl1* is normally expressed in all motoneurons, and the experimentally induced expression of *Lhx3*, is sufficient to generate the MMCm fate (Sharma et al. 2000).

An integral part of motoneuron subtype identity is the extension of the axon in the correct trajectory and to the appropriate target. The LMCl subtype of motoneurons normally project to the dorsal limb muscles, and these neurons selectively express *Lhx1*. In the *Lhx1* mutant, the LMCl axons show no preference for the dorsal versus ventral limb musculature (Kania et al. 2000). One hypothesis is that the "LIM code" confers differential responses to various chemoattractants or chemorepellants (Varela-Echavarria et al. 1997) and in the absence of *Lhx1*, the selectivity of the LMCl neurons for dorsal muscle targets is lost (Kania et al. 2000). A mechanism for this function of Lhx1 has recently been identified. Axon outgrowth is inhibited by the interaction of receptor tyrosine kinase EphA4 with its ligand ephrin-A. In chick embryos, Kania and Jessell (2003) showed that electroporation of *Lim1* (*Lhx1*) upregulates EphA4 in LMC neurons, while electroporation of Isl1 downregulates EphA4 protein levels in these cells. This suggests a scenario where normally, the presence of Lhx1 in the LMCl neurons would elevate the level of EphA4 levels, while presence of Isl1 in the LMCm neurons would repress EphA4 levels. These authors further showed that LIM-HD gene Lmx1b is responsible for the enhanced concentration of net free ephrin-A in the ventral musculature. Thus, the EphA4 rich LMCl axons are inhibited from innervating this tissue, whereas the LMCm neurons, having low EphA levels are able to innervate it (Kania & Jessell 2003). These studies provide the first conclusive evidence showing that the "LIM code" of a particular motor neuron subtype is *directly responsible* for regulating molecules that influence axon pathfinding.

In summary, the "LIM code" in the ventral spinal cord, and the complex interactions revealed in the hexameric model of LIM gene action, greatly extend our understanding of how these genes function. Furthermore, the role of LIM genes in the specification of neuronal identity and in the regulation of axon pathfinding in the spinal cord motivates an examination of these genes in the cerebral cortex, a structure in which their roles are not as well understood.

LIM Genes in the Cerebral Cortex

The cerebral cortex develops from the telencephalon, which consists of paired telencephalic vesicles that arise at the most rostral portion of the embryonic neural tube. Morphological changes in the telencephalon result from proliferation and migration of the neuroepithelial cells. Postmitotic neurons are generated in the ventricular zone and migrate to different sites (reviewed in McConnell 1991). By embryonic day (E) 10.5 in the mouse, the dorsal midline region of the telencephalon has invaginated, giving rise to the paired telencephalic vesicles. The medial aspect of these vesicles contains the presumptive hippocampal primordium, as well as a signaling center called the cortical hem, rich in signaling molecules of the Wnt and the BMP families (Grove et al. 1998). The neocortex arises from the dorsal and lateral portions of the telencephalic vesicles (figure 7). The ventral region of the telencephalon gives rise to the striatum, a non-cortical structure. Cortical neurons born from the medial, dorsal, and lateral portions of the telencephalic ventricular zone migrate outward to establish the post mitotic cortical plate (reviewed in McConnell 1995). The ventral telencephalon also contributes a population of neurons to the cortex that migrate to reside in the

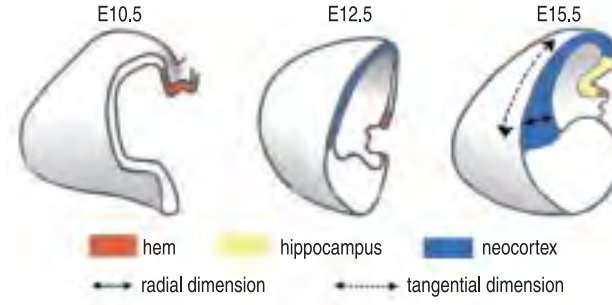


Figure 7. Development of the telencephalon in the mouse embryo from early to mid gestational stages. At early stages, a signaling center (red) is identified in the medial portion of the telencephalic neuroepithelium, while the remaining portion of the dorsal telencephalon is the prospective cerebral cortex. By E12.5, the prospective cortical neuroepithelium has a distinct medial (yellow) and lateral (blue) portion, which will form the hippocampus and the neocortex respectively, at later stages. Different regions of the neuroepithelium give rise to distinct cortical areas along the tangential axis (dashed arrows). Along the radial axis (solid arrows), neurons are assigned a particular layer-identity depending on where they reside in the postmitotic cerebral cortex.

cortical plate (Lavdas et al. 1999, Anderson et al. 2001). After neuronal migration is complete, the cortical plate displays the characteristic layered organization of the mature cerebral cortex. Each cortical layer has cells with distinct features and patterns of connectivity. Specification of neuronal identity in the cerebral cortex is therefore a twofold problem: specification of regional identity along the tangential dimension, as well as specification of layer identity along the radial dimension (figure 7).

LIM Genes in Patterning the Embryonic Telencephalon

Several members of the LIM gene family are expressed in the developing forebrain. It is not clear, however, if the LIM code of the spinal cord is conserved in the telencephalon. Nonetheless, studies in knockout mice have revealed important roles for these genes in various aspects of telencephalic development. *Lhx2-/-* mice display hypoplasia in the telencephalon (Porter et al. 1997). *Lhx5-/-* mice show defective hippocampal development, in proliferation of precursors as well as in migration of postmitotic neurons (Zhao et al. 1999). In maturity, the hippocampal neuroanatomy in these animals is highly disorganized, possibly underlying the severe learning and memory defects that have been reported in these mice (Paylor et al. 2001). *Lhx6* and *Lhx7* are not expressed in the dorsal telencephalon, but are present in specific regions of the ventral telencephalon, where they are implicated in development of distinct subtypes of striatal interneurons and cortical interneurons that originate from the ventral telencephalon (Lavdas et al. 1999, Marin et al. 2000). Finally, a spontaneous autosomal recessive mutation called *dreher* was identified as a mutation in *Lmx1a*, a LIM-HD gene that is expressed in the roof plate of the CNS (Millonig et al. 2000). The homozygous mutants (dr^{J}/dr^{J}) show aberrantly positioned neurons in the cerebral cortex, hippocampus and cerebellum (Costa et al. 2001). In the cortex, this defect is a result of over-migration of neurons destined for layer II, and is correlated with a disruption of the glial limiting membrane of the radial glia that guide neuronal migration (Costa et al. 2001).

Although these experiments indicate several LIM-HD have major roles in telencephalic development, they do not directly serve to highlight how these genes function. How do studies in the vertebrate spinal cord or the *apterous* model bear on LIM-HD genes in the forebrain?

Lhx2 regulates the Formation of the Cortical hem

The *Lhx2* mutant brain, previously reported to have a shrunken cortex (Porter et al. 1997), presented an opportunity to explore the role of this gene in cortical development. We hypothesized that the cortical hem, a signaling center thought to regulate the growth and patterning of the cortex (Grove et al. 1998, Lee et al. 2000), may be defective or deficient in this mutant. Examination of the *Lhx2* mutant revealed an unexpected result: the hem was greatly expanded, rather than reduced; what had been previously interpreted as shrunken cortex in this mutant is in fact enlarged hem tissue, while the cortex itself is almost entirely absent (figure 8; Bulchand et al. 2001, Monuki et al. 2001). These results reveal a crucial role for Lhx2 in regulating the boundary between the hem and the

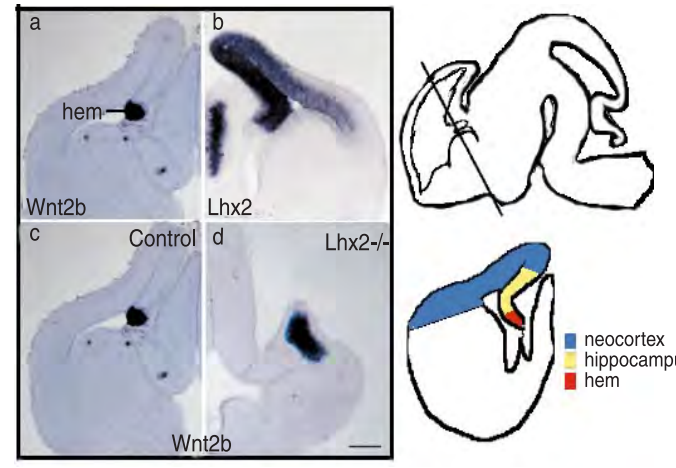


Figure 8. *Wnt2b* expression identifies an expanded cortical hem in the Lhx2-/- brain.
a-c: Coronal sections of control mouse brains, d: coronal section of Lhx2-/- brain. At E12.5, *Wnt2b* is expressed only in the hem of control sections **(a, c)**, while *Lhx2* expression excludes the hem **(b)**. In the Lhx2-/- brain, the Wnt2b expressing region appears greatly expanded **(d).** Scale bar is 300 microns.
Modified and reprinted from Mechanisms of Development, Vol 100, Bulchand, S. et al. LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem., 165-75, Copyright (2001), with permission from Elsevier.

presumptive cortex. Consistent with such a role, we find *Lhx2* expression in normal brains to be present in the entire cortical neuroepithelium, both lateral and medial portions, but excluded from the cortical hem (figure 8; Bulchand et al. 2001). We propose that Lhx2 function is normally required for a cortical identity, without which the cells appear to take the identity of the hem (Bulchand et al. 2001). Such a role in specifying cells on one side of a boundary has parallels with that of *apterous* in the dorsal cell specification of the *Drosophila* wing.

From what is known about the functional modulation of the LIM-HD genes, it seems clear that any role they play in patterning a complex structure like the telencephalon will be subject to extensive regulation, and that the same genes may subserve different functions at different stages of growth and maturation. In order to gain an insight into how this gene family may influence the development of the cortex, it is important to understand what co-factors and competitors are present and available to the LIM-HD genes during critical developmental events. A similar analysis of the expression patterns of these genes in the developing spinal cord laid the ground-work for understanding of their role in specifying motoneuron and interneuron identity.

Dynamic Expression of LIM Genes and Co-factors in the Embryonic Cerebral Cortex

The above considerations prompted us to do an exhaustive examination of the spatial and temporal patterns of the LIM-HD, *Lmo*, and *Clim* genes in the telencephalon, by *in situ* hybridization (Bulchand et al. 2003). The mouse telencephalon expresses 12 LIM-HD, 4 *Lmo* and 2 *Clim* genes during development (Thor et al. 1991, Foroni et al. 1992, Fujii et al. 1994, Bach et al. 1997, Porter et al. 1997, Sheng et al. 1997, Grigoriou et al. 1998, Kitanaka et al. 1998, Bertuzzi et al. 1999, Hermanson et al. 1999, Retaux et al. 1999, Millonig et al. 2000, Bulchand et al. 2003). Quite obviously, there is scope for widespread regulation of the LIM-HD transcription factors, both spatially and temporally, which can then influence downstream mechanisms. We selected three developmental stages for our studies: First, an early embryonic stage, E12.5, when the neuroepithelium is highly proliferative, and is clearly divided into the prospective cortex and a signaling center, the cortical hem; second, a mid to late embryonic stage (E15.5-E17.5), when postmitotic neurons are migrating to the cortical plate and layer formation is in progress; finally, postnatal stages, when the laminar organization of the cortex is complete.

Embryonic Expression

At early embryonic stages, several LIM-HD genes demarcate the boundary between the hem and the adjacent cortical tissue. *Lmx1a* is expressed selectively in the hem, while *Lhx2 and Lhx9* are expressed in different patterns in the cortical neuroepithelium (figure 9a-c; Retaux et al. 1999, Failli et al. 2002, Bulchand et al. 2003). *Lhx9* expression is

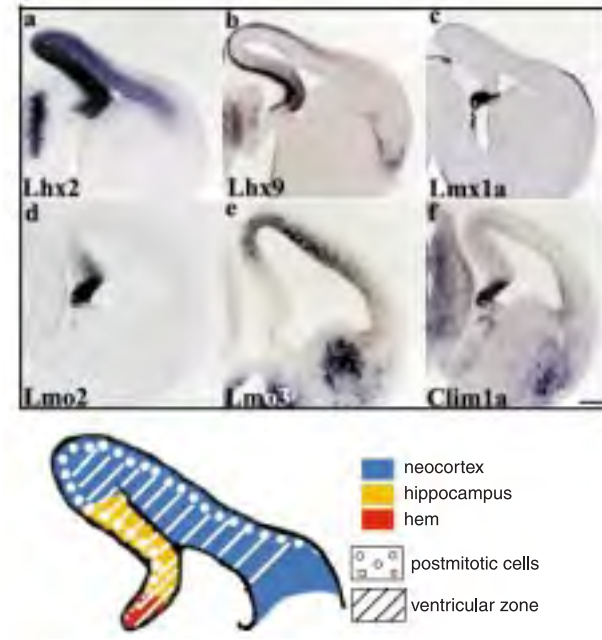


Figure 9. Early embryonic expression of Lim genes and cofactors in the telencephalic neuroepithelium. a–f: Coronal sections of embryonic day 12.5 mouse brain. *Lhx2* is found in the neuroepithelium of the hippocampus and the lateral cortex, but excludes the hem **(a)**; *Lhx9* is expressed in the postmitotic cells of the entire medial and lateral neuroepithelium, and also in the ventricular zone of the hippocampal primordium **(b)**; *Lmx1a* **(c)** *Lmo2* expression in the cortical hem continues into the adjacent hippocampal primordium **(d);** *Lmo3* expression is detected in the ventricular zone of the most of the lateral, and part of the medial neuroepithelium **(e)**. and *Clim1a* **(f)** are expressed in the cortical hem. Scale bar is 300 microns. From Developmental Dynamics, Vol. **226**, pg 460-469. Bulchand et al. Dynamic Spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex. Copyright (2003) Wiley-Liss, Inc. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

found in the postmitotic cells of the entire dorsal telencephalon, including the lateral and medial cortex, and continuing into the cortical hem. In the hippocampal primordium, the proliferating cells also display *Lhx9* expression (figure 9b; Bulchand et al. 2003). *Lhx2* is expressed in the entire lateral and medial cortical neuroepithelium (figure 9a; Bulchand et al. 2003). Together, these LIM-HD genes may regulate cell identity or axon pathfinding in a combinatorial fashion in various regions of the neuroepithelium where they are expressed.

Of particular interest is the fact that the apparently uniform domain of *Lhx2* expression in the cortex is subdivided by the differential expression of the *Lmo* genes. *Lmo2* and *Lmo3* show a complementary expression, with *Lmo2* detected in part of the medial cortex, tapering off dorsally, while *Lmo3* expression picks up where Lmo2 diminishes, and continues more strongly in the lateral neuroepithelium (figure 9d,e; Bulchand et al. 2003). If, according to the *Apterous* model, the Lmo proteins function as competitors to Lhx2, then Lmo2 and Lmo3 could potentially regulate Lhx2 function differentially, in their respective areas of expression. Such a possibility would provide a mechanism for a distinct role Lhx2 in the medial cortex versus the lateral cortex. Among the co-factors, Clim1a is presumably not available to Lhx2, since *Clim1a* expression is restricted to the hem (figure 9f; Bulchand et al. 2003). *Clim2*, in contrast, is ubiquitously expressed (not shown; Bulchand et al. 2003).

At mid to late embryonic stages, a new feature emerges in the expression patterns of several *Lmo* genes and *Clim1a*, where they demarcate cortical area boundaries. The ventrally located olfactory cortex, the caudally located visual cortex, the dorsolaterally located sensorimotor cortex, or the boundaries between these regions are delineated by the expression of various combinations of these genes (figure 10a-d; Bulchand et al. 2003). It is curious that none of the LIM-HD genes exhibit similar patterns of expression with respect to cortical area boundaries, suggesting that the Lmo and Clim1a proteins may act in novel, LIM-HD independent mechanisms.

Postnatal Expression

Postnatally, the LIM and Clim genes exhibit distinct laminar patterns in the cortex. *Lhx2* remains restricted to the superficial layers of the cortex, while *Clim1a* is expressed intensely in a deeper layer, in a strip of cells that does not overlap with the *Lhx2* expressing layers (figure 11a-c; Bulchand et al. 2003). *Lmo3* is expressed in two bands: a narrow band within the superficial layers, which overlaps with *Lhx2* expression, and in a broader band in the deep layers, complementary to that of *Lhx2* (figure 11d-f; Bulchand et al. 2003).

The layer specific expression of *Lhx2* may be relevant in two different contexts. Firstly, it may

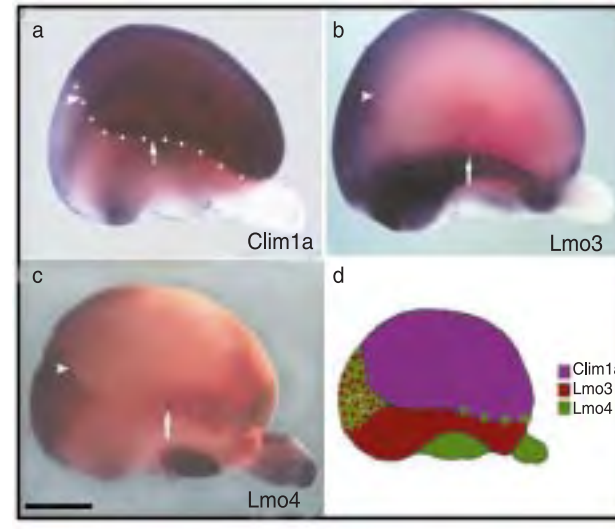
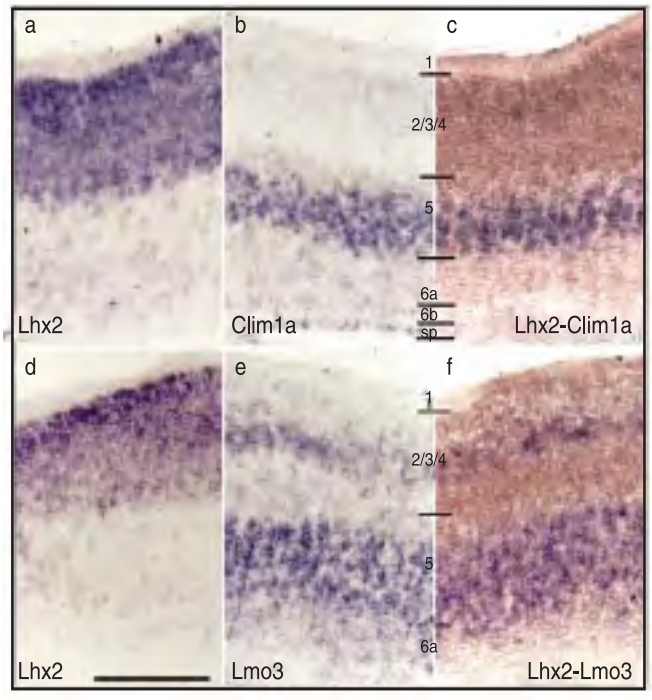


Figure 10. a–d: Regional expression patterns of Lim genes and cofactors in intact telencephalic hemispheres. a–d, lateral views of mid-late embryonic telencephalic hemispheres. *Clim1a* is expressed in a continuous region that covers much of the rostral and dorsal portions of the hemisphere (dotted line, **a**). The caudal and ventral regions are excluded, defining two boundaries (arrow and arrowhead, **a**). *Lmo3* (**b**), and *Lmo4* (**c**) expression appears to recognize similar boundaries (arrows and arrowheads). *Lmo3* expression is complementary to *Clim1a*, being present in the olfactory cortex ventrally, and in the visual cortex caudal to the *Clim1a* expressing region **(b)**. *Lmo4* is expressed in the visual cortex, and in a band along the *Lmo3-Clim1a* expression boundary **(**arrow**, c)**. For both lateral and medial views, rostral is to the right. The expression patterns are schematized in the adjacent diagram (**d**). sm, sensorimotor cortex; olf, olfactory cortex; vis, visual cortex. Scale bar is 1mm. From ***Developmental Dynamics,*** Vol. **226**, pg 460-469. Bulchand et al. Dynamic Spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex. Copyright (2003) Wiley-Liss, Inc. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.



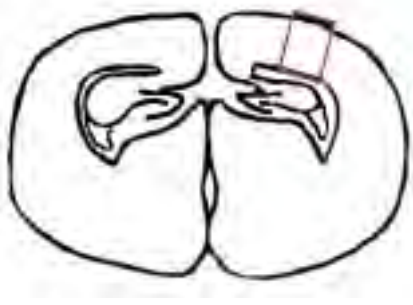


Figure 11. Layer-specific expression patterns of Lim genes and cofactors in the postnatal cerebral cortex. a–f: Coronal sections from postnatal day 8 brain. a–c and d–f are sets of consecutive sections. *Lhx2* is expressed strongly in layers 2/3/4 and in a superficial portion of layer 5 **(a,d)**. *Clim1a* is expressed in a deeper portion of layer 5**(b)**. *Lhx2* and *Clim1a* expression are complementary to each other, visualized by two-color *in-situ* hybridization (brown, *Lhx2*; purple, *Clim1a*) **(c)**. *Lmo3*-positive cells are seen in a narrow band of cells in the superficial layers, where they overlap with *Lhx2* expression. *Lmo3* is also expressed in a broad zone in the deep layers, including most of layer 5 and 6a **(e)**. This zone of expression complementary to *Lhx2* expression (brown, *Lhx2*; purple, *Lmo3*) **(f)**. Scale bar is 300 microns. From ***Developmental Dynamics,*** Vol. **226**, pg 460-469. Bulchand et al. Dynamic Spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex. Copyright (2003) Wiley-Liss, Inc. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

indicate a role for Lhx2 in the specification of neurons in particular cortical layers. Alternatively, it may also be important in establishing axonal connections between cortex and the thalamus, a function that depends on precise layer specification in the cortex (Bachy et al. 2002).

In summary, we find dynamic expression of several LIM-HD, *Lmo*, and *Clim* genes at different stages of development in the cerebral cortex. Not only do some LIM genes have already established, critical roles in the development of the cortex, but they are also expressed in highly specific and unique spatio-temporal patterns. These expression patterns mark significant features of cortical maturation, distinguishing between proliferating versus postmitotic cells, different cortical areas, as well as distinct layers of postmitotic cortex.

Perspectives

LIM-HD genes display a great diversity of expression in a given species, and at the same time maintain a remarkable evolutionary conservation of expression as well as function across species. *ap* and its mouse ortholog, *Lhx2*, are expressed in the corresponding structures in *Drosophila* and mouse, such as the brain, nerve cord/spinal cord, olfactory organ, eye, and limb. In the *Drosophila* wing, the human ortholog of *ap*, *hLhx2*, can effectively rescue *ap* mutant phenotypes (Rincon-Limas et al. 1999), suggesting a similar mechanism of action of these proteins at the molecular level. Ap regulates both dorsal specification as well as outgrowth of the wing, and a striking parallel is found in the chick limb, where cLhx2 and cLmx1 share these roles, regulating outgrowth and dorsal cell fate specification, respectively (Rodriguez-Esteban et al. 1998). Another parallel is found in the *Drosophila* VNC and the vertebrate spinal cord, where a combinatorial "LIM code" regulates neuronal pathfinding.

However, compared with *Drosophila,* much less is known about downstream targets that mediate their role in cell identity specification in vertebrates. In the vertebrate limb, Lhx2 regulates *radical fringe*, a homolog of *Drosophila fng*, but it is not known whether Dl or Ser homologs are also regulated by Lhx2 (Rodriguez-Esteban et al. 1998). Downstream targets of cLmx1 are as yet unknown.

In vertebrate systems, the molecules that mediate LIM-HD action in the specification of cell identity are largely unknown. In contrast, a mechanism by which LIM-HD genes regulate axonal pathfinding has recently been discovered in the chick spinal cord, mediated by the regulation of EphA: Ephrin-A interactions in motoneurons and in the limb mesenchyme (Kania & Jessell 2003). It would be important to examine if LIM-HD regulation of axonal pathfinding in *Drosophila* is also mediated by the corresponding homolog, Dephrin. Also, other molecules implicated in pathfinding functions in motoneurons, such as Netrins and Semaphorins (Varela-Echavarria et al. 1997), might also mediate the role of LIM-HD genes in these processes.

The tetrameric model proposed for the mechanism of Ap action in the *Drosophila* wing appears to be a requirement of LIM-HD mediated transcriptional activity in this system. However, there is clear evidence in the mouse and chick spinal cord of a more complex set of interactions than those in the Ap model. Furthermore, in *Drosophila* itself, it appears that the tetrameric model for Ap action does not account for all aspects of Ap function e.g. swapping LIM domains between Ap and other *Drosophila* LIM-HD genes rescues the *ap* wing phenotype, but not pathfinding defects in Ap-expressing neurons. This suggests that different LIM domains may have specific interactions that generate distinct functions, or that the Clim proteins may have a more complex role than merely forming a tetrameric complex. Indeed, the Ssdp family of proteins has recently been found to interact with Clim molecules at a site independent of the dimerization domain or the LIM-interaction domain, and disruption of *ssdp* had been reported to mimic *ap* and *chip* phenotypes (Chen et al. 2002, van Meyel et al. 2003). In *Drosophila*, there is only one known Clim homolog (Chip), whereas in the mouse brain, there is one ubiquituously expressed Clim protein, Clim2, and one extremely restricted member, Clim1a. The differences in function between these, if any, have yet to be determined, but may reveal selective interactions with specific LIM-HD proteins or other interactors, and provide a functional basis for the diversity of LIM domains.

In the embryonic and mature cerebral cortex, the role of LIM-HD, *Lmo*, and *Clim* genes remains largely unexplored. A combinatorial expression pattern in the thalamus (Nakagawa & O'Leary 2001) has motivated a hypothesis for a role of LIM-HD genes in thalamocortical pathfinding (Nakagawa & O'Leary 2001, Bachy et al. 2002). Studies of mice in which selected LIM-HD genes have been disrupted have revealed roles for some LIM-HD genes in the development of specific portions of the cortex or hippocampus, but these mutants have not been examined for pathfinding defects. Furthermore, the mechanism of action of these genes is unclear. It is essential to determine, for example, whether any LIM-HD and Lmo proteins genes bind more efficiently to Clim1a over Clim2, and whether a particular Lmo protein is a specific competitor to certain LIM-HD genes. It is also not known whether LIM-HD proteins that are expressed in overlapping regions in the cortex bind to each other, possibly participating in multimeric complexes with Clim molecules, as in the vertebrate spinal cord. Furthermore, several *Lmo* genes as well as *Clim1a* are expressed in sites where they do not overlap with any LIM-HD gene expression, suggesting a role for these molecules in the cortex that is independent of LIM-HD proteins. Such a role has been observed in other systems. In *Drosophila* segmentation, Chip interacts with diverse homeodomain proteins via residues distinct from those that interact with LIM domains (Torigoi et al. 2000). Additionally, there is evidence of direct interactions between Lmo molecules and members of the basic helix-loop-helix family of transcription factors, in neurogenesis, and in T cell leukemia (Wadman et al. 1994, Valge-Archer et al. 1994, Bao et al. 2000). Similarly, Lmo proteins may act in complexes to regulate gene expression independent of LIM-HD proteins in the developing cerebral cortex. Such roles would add a new dimension to existing models of LIM gene function.

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References

Adams K, Maida J, Golden J and Riddle R 2000 The transcription factor Lmx1b maintains Wnt1 expression within the isthmic organizer; *Development* **127** 1857-1867

Agulnick A, Taira M, Breen J, Tanaka T, Dawid I and Westphal H 1996 Interactions of the LIM-domain-binding factor Ldb1 with LIM homeodomain proteins; *Nature* **384** 270-272

Ahlgren U, Pfaff S, Jessell T, Edlund T and Edlund H 1997 Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells; *Nature* **385** 257-260

Allan D, Pierre S, Miguel-Aliaga I and Thor S 2003 Specification of Neuropeptide Cell Identity by the Integration of Retrograde BMP Signaling and a Combinatorial Transcription Factor Code; *Cell* **113** 73-86

Altaratz M, Applebaum S, Richard D, Gilbert L and Segal D 1991 Regulation of juvenile hormone synthesis in wild-type and apterous mutant Drosophila; *Mol. Cell Endocrinol.* **81** 205-216

Altun-Gultekin Z, Andachi Y, Tsalik E, Pilgrim D, Kohara Y and Hobert O 2001 A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in *C. elegans; Development* **128** 1951-1969

Anderson S, Marin O, Horn C, Jennings K and Rubenstein J 2001 Distinct cortical migrations from the medial and lateral ganglionic eminences; *Development* **128** 353-363

Aurelio O, Boulin T and Hobert O 2003 Identification of spatial and temporal cues that regulate postembryonic expression of axon maintenance factors in the C.elegans ventral nerve cord; *Development* **130** 599-610

Bach I, Carriere C, Ostendorff H, Andersen B and Rosenfeld M 1997 A family of LIM domain-associated cofactors confer transcriptional synergism between LIM and Otx homeodomain proteins; *Genes Dev.* **11** 1370-1380

Bachy I, Failli V and Retaux S 2002 A LIM-homeodomain code for development and evolution of forebrain connectivity; *Neuroreport* **13** A23-27

Bao J, Talmage D, Role L and Gautier J 2000 Regulation of neurogenesis by interactions between HEN1 and neuronal LMO proteins; *Development* **127** 425-435

Benveniste R, Thor S, Thomas J and Taghert P 1998 Cell type-specific regulation of the *Drosophila* FMRF-NH2 neuropeptide gene by Apterous, a LIM homeodomain transcription factor; *Development* **125** 4757-4765

Bertuzzi S, Porter F, Pitts A, Kumar M, Agulnick A, Wassif C and Westphal H 1999 Characterization of Lhx9, a novel LIM/homeobox gene expressed by the pioneer neurons in the mouse cerebral cortex; *Mech. Dev.* **81** 193-198

Birk O, Casiano D, Wassif C, Cogliati T, Zhao L, Zhao Y, Grinberg A, Huang S, Kreidberg J, Parker K, Porter F and Westphal H 2000 The LIM homeobox gene Lhx9 is essential for mouse gonad formation; *Nature* **403** 909-913

Blair S 1993 Mechanisms of compartment formation: evidence that non-proliferating cells do not play a critical role in defining the D/V lineage restriction in the developing wing of Drosophila; *Development* **119** 339-351

_____, Brower D, Thomas J and Zavortink M 1994 The role of apterous in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of *Drosophila; Development* **120** 1805-1815

Bourgouin C, Lundgren S and Thomas J 1992 Apterous is a *Drosophila* LIM domain gene required for the development of a subset of embryonic muscles; *Neuron.* **9** 549-561

Breen J J, Agulnick A D, Westphal H and Dawid I B 1998 Interactions between LIM domains and the LIM domain-binding protein Ldb1; *J. Biol. Chem.* **273** 4712-4717

Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell T, Rubenstein J and Ericson J 1999 Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling; *Nature* **398** 622-627

_____, Pierani A, Jessell T and Ericson J 2000 A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube; *Cell* **101** 435-445

Bulchand S, Grove E, Porter F and Tole S 2001 LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem; *Mech. Dev.* **100** 165-175

_____, Subramanian L and Tole S 2003 Dynamic spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex; *Dev. Dyn.* **226** 460-469

Burrill J, Moran L, Goulding M and Saueressig H 1997 PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development; *Development* **124** 4493-4503

Cassata G, Kagoshima H, Andachi Y, Kohara Y, Durrenberger M, Hall D and Burglin T 2000 The LIM homeobox gene ceh-14 confers thermosensory function to the AFD neurons in *Caenorhabditis elegans; Neuron.* **25** 587-597

Chen H, Ovchinnikov D, Pressman C, Aulehla A, Lun Y and Johnson R 1998a Multiple calvarial defects in lmx1b mutant mice; *Dev. Genet.* **22** 314-320

_____, Lun Y, Ovchinnikov D, Kokubo H, Oberg K, Pepicelli C, Gan L, Lee B and Johnson R 1998b Limb and kidney defects in Lmx1b mutant mice suggest an involvement of LMX1B in human nail patella syndrome; *Nat. Genet.* **19** 51-55

Chen L, Segal D, Hukriede N, Podtelejnikov A, Bayarsaihan D, Kennison J, Ogryzko V, Dawid I and Westphal H 2002 Ssdp proteins interact with the LIM-domain-binding protein Ldb1 to regulate development; *Proc. Natl. Acad. Sci. U S A* **99** 14320-14325

Cohen B, McGuffin M, Pfeifle C, Segal D and Cohen S 1992 Apterous, a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins; *Genes Dev.* **6** 715-729

Costa C, Harding B and Copp A 2001 Neuronal migration defects in the Dreher (Lmx1a) mutant mouse: role of disorders of the glial limiting membrane; *Cereb. Cortex* **11** 498-505

Curtiss J and Heilig J S 1995 Establishment of *Drophila* imaginal precursor cells is controlled by the Arrowhead gene; *Development* **121** 3819-3829

_____ and _____ 1997 Arrowhead encodes a LIM homeadomain protein that distinguishes subsets of *Drosophila* imaginal cells; *Dev. Biol.* **190** 129-141

de Celis J and Bray S 1997 Feed-back mechanisms affecting Notch activation at the dorsoventral boundary in the *Drosophila* wing; *Development* **124** 3241-3251

Diaz-Benjumea F and Cohen S 1993 Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila; Cell* **75** 741-752

_____ and Cohen S 1995 Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing; *Development* **121** 4215-4225

Duggan A, Ma C and Chalfie M 1998 Regulation of touch receptor differentiation by the *Caenorhabditis elegans* mec-3 and unc-86 genes; *Development* **125** 4107-4119

Echelard Y, Epstein D, St-Jacques B, Shen L, Mohler J, McMahon J and McMahon A 1993 Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity; *Cell* **75** 1417-1430

Ericson J, Thor S, Edlund T, Jessell T and Yamada, T 1992 Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1; *Science* **256** 1555-1560

_____, Muhr J, Placzek M, Lints T, Jessell T and Edlund T 1995 Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube; *Cell* **81** 747-756

Ericson J, Morton S, Kawakami A, Roelink H and Jessell T 1996 Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity; *Cell* **87** 661-673

_____, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell T and Briscoe J 1997 Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling; *Cell* **90** 169-180

Failli V, Bachy I and Retaux S 2002 Expression of the LIM-homeodomain gene Lmx1a (dreher) during development of the mouse nervous system; *Mech. Dev.* **118** 225-228

Fernandez-Funez P, Lu C, Rincon-Limas D, Garcia-Bellido A and Botas J 1998 The relative expression amounts of apterous and its co-factor dLdb/Chip are critical for dorso-ventral compartmentalization in the *Drosophila* wing; *EMBO J.* **17** 6846-6853

Fleming R, Gu Y and Hukriede N 1997 Serrate-mediated activation of Notch is specifically blocked by the product of the gene fringe in the dorsal compartment of the *Drosophila* wing imaginal disc; *Development* **124** 2973-2981

Foroni L, Boehm T, White L, Forster A, Sherrington P, Liao X, Brannan C, Jenkins N, Copeland N and Rabbitts T 1992 The rhombotin gene family encode related LIM-domain proteins whose differing expression suggests multiple roles in mouse development; *J. Mol. Biol.* **226** 747-761

Fujii T, Pichel J, Taira M, Toyama R, Dawid I and Westphal H 1994 Expression patterns of the murine LIM class homeobox gene lim1 in the developing brain and excretory system; *Dev. Dyn.* **199** 73-83

Ghazi A, Anant S and VijayRaghavan K 2000 Apterous mediates development of direct flight muscles autonomously and indirect flight muscles through epidermal cues; *Development* **127** 5309-5318

Grigoriou M, Tucker A, Sharpe P and Pachnis V 1998 Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development; *Development* **125** 2063-2074

Grove E, Tole S, Limon J, Yip L and Ragsdale C 1998 The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice; *Development* **125** 2315-2325

Gupta B, Wang M and Sternberg P 2003 The *C. elegans* LIM homeobox gene lin-11 specifies multiple cell fates during vulval development; *Development* **130** 2589-2601

Helms A and Johnson J 2003 Specification of dorsal spinal cord interneurons; *Curr. Opin. Neurobiol.* **13** 42-49

Hermanson O, Sugihara T and Andersen B 1999 Expression of LMO-4 in the central nervous system of the embryonic and adult mouse; *Cell. Mol. Biol. (Noisy-le-grand)* **45** 677-686

Herzig M, Thor S, Thomas J, Reichert H and Hirth F 2001 Expression and function of the LIM homeodomain protein Apterous during embryonic brain development of *Drosophila; Dev. Genes. Evol.* **211** 545-554

Hobert O, Mori I, Yamashita Y, Honda H, Ohshima Y, Liu Y and Ruvkun G 1997 Regulation of interneuron function in the *C. elegans* thermoregulatory pathway by the ttx-3 LIM homeobox gene; *Neuron.* **19** 345-357

Hobert O, D'Alberti T, Liu Y and Ruvkun G 1998 Control of neural development and function in a thermoregulatory network by the LIM homeobox gene lin-11; *J. Neurosci.* **18** 2084-2096

_____, Tessmar K and Ruvkun G 1999 The *Caenorhabditis elegans* lim-6 LIM homeobox gene regulates neurite outgrowth and function of particular GABAergic neurons; *Development* **126** 1547-1562

Irvine K and Wieschaus E 1994 Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development; *Cell* **79** 595-606

Jurata L W, Kenny D A and Gill G N 1996 Nuclear LIM interactor, a rhombotin and LIM homeodomain interacting protein, is expressed early in neuronal development; *Proc. Natl. Acad. Sci. U S A* **93** 11693-11698

_____ and Gill G N 1997 Functional analysis of the nuclear LIM domain interactor NLI; *Mol. Cell. Biol.* **17** 5 688-5698

_____, Pfaff S L, Gill G N 1998 The nuclear LIM domain interactor NLI mediates homo-and heterodimerization of LIM domain transcription factors; *J. Biol. Chem.* **273** 3152-3157

Kania A, Johnson R and Jessell T 2000 Coordinate roles for LIM homeobox genes in directing the dorsoventral trajectory of motor axons in the vertebrate limb; *Cell* **102** 161-173

_____ and Jessell T 2003 Topographic motor projections in the limb imposed by LIM homeodomain protein regulation of Ephrin-A:EphA interactions; *Neuron.* **38** 581-596

Kim J, Irvine K and Carroll S 1995 Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing *Drosophila* wing; *Cell* **82** 795-802

Kinder S, Tsang T, Ang S, Behringer R and Tam P 2001 Defects of the body plan of mutant embryos lacking Lim1, Otx2 or Hnf3beta activity; *Int. J. Dev. Biol.* **45** 347-355

Kitanaka J, Takemura M, Matsumoto K, Mori T and Wanaka A1998 Structure and chromosomal localization of a murine LIM/homeobox gene, Lhx8; *Genomics* **49** 307-309

Klein T and Arias A 1998 Interactions among Delta, Serrate and Fringe modulate Notch activity during *Drosophila* wing development; *Development* **125** 2951-2962

Lavdas A, Grigoriou M, Pachnis V and Parnavelas J 1999 The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex; *J. Neurosci.* **19** 7881-7888

Lee S, Tole S, Grove E and McMahon A 2000 A local Wnt-3a signal is required for development of the mammalian hippocampus; *Development* **127** 457-467

_____ and Pfaff S 2001 Transcriptional networks regulating neuronal identity in the developing spinal cord; *Nat. Neurosci.* **4** 1183-1191

Liem K J, Tremml G, Roelink H and Jessell J 1995 Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm; *Cell* **82** 969-979

_____, _____ and Jessell T 1997 A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord; *Cell* **91** 127-138

Lilly B, O'Keefe D, Thomas J and Botas J 1999 The LIM homeodomain protein dLim1 defines a subclass of neurons within the embryonic ventral nerve cord of Drosophila; *Mech. Dev.* **88** 195-205

Lundgren S, Callahan C, Thor S and Thomas J 1995 Control of neuronal pathway selection by the *Drosophila* LIM homeodomain gene apterous; *Development* **121** 1769-1773

Marin O, Anderson S and Rubenstein J 2000 Origin and molecular specification of striatal interneurons; *J. Neurosci.* **20** 6063-6076

Marti E, Takada R, Bumcrot D, Sasaki H and McMahon A 1995 Distribution of sonic hedgehog peptides in the developing chick and mouse embryo; *Development* **121** 2537-2547

McConnell S 1991 The generation of neuronal diversity in the central nervous system; *Annu. Rev. Neurosci.* **14** 269-300

_____ 1995 Constructing the cerebral cortex: neurogenesis and fate determination; *Neuron.* **15** 761-768

Milan M, Diaz-Benjumea F and Cohen S 1998 Beadex encodes an LMO protein that regulates Apterous LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function; *Genes. Dev.* **12** 2912-2920

_____ and Cohen S 1999 Regulation of LIM homeodomain activity *in vivo:* a tetramer of dLDB and apterous confers activity and capacity for regulation by dLMO; *Mol. Cell* **4** 267-273

_____ and _____ 2000 Temporal regulation of apterous activity during development of the *Drosophila* wing; *Development* **127** 3069-3078

_____, Weihe U, Perez L and Cohen S 2001a The LRR proteins capricious and Tartan mediate cell interactions during DV boundary formation in the *Drosophila* wing; *Cell* **106** 785-794

_____, Weihe U, Tiong S, Bender W and Cohen S 2001b msh specifies dorsal cell fate in the *Drosophila* wing; *Development* **128** 3263-3268

_____ and Cohen S 2003 A re-evaluation of the contributions of Apterous and Notch to the dorsoventral lineage restriction boundary in the *Drosophila* wing; *Development* **130** 553-562

Millonig J, Millen K and Hatten M 2000 The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS; *Nature* **403** 461-469

Miner J, Morello R, Andrews K, Li C, Antignac C, Shaw A and Lee B 2002 Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation; *J. Clin. Invest.* **109** 1065-1072

Monuki E, Porter F and Walsh C 2001 Patterning of the dorsal telencephalon and cerebral cortex by a roof plate-Lhx2 pathway; *Neuron.* **32** 591-604

Morcillo P, Rosen C, Baylies M and Dorsett D 1997 Chip, a widely expressed chromosomal protein required for segmentation and activity of a remote wing margin enhancer in *Drosophila; Genes. Dev.* **11** 2729-2740

Nakagawa Y and O'Leary D 2001 Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus; *J. Neurosci.* **21** 2711-2725

Newman A, Acton G, Hartwieg E, Horvitz H and Sternberg P 1999 The lin-11 LIM domain transcription factor is necessary for morphogenesis of *C. elegans* uterine cells; *Development* **126** 5319-5326

Ng M, Diaz-Benjumea F, Vincent J, Wu J and Cohen S 1996 Specification of the wing by localized expression of wingless protein; *Nature* **381** 316-318

O'Keefe D, Thor S and Thomas J 1998 Function and specificity of LIM domains in *Drosophila* nervous system and wing development; *Development* **125** 3915-3923

_____ and Thomas J 2001 *Drosophila* wing development in the absence of dorsal identity; *Development* **128** 703-710

Osumi N, Hirota A, Ohuchi H, Nakafuku M, Iimura T, Kuratani S, Fujiwara M, Noji S and Eto K 1997 Pax-6 is involved in the specification of hindbrain motor neuron subtype; *Development* **124** 2961-2972

Panin V, Papayannopoulos V, Wilson R and Irvine K1997 Fringe modulates Notch-ligand interactions; *Nature* **387** 908-912

Paylor R, Zhao Y, Libbey M, Westphal H and Crawley J 2001 Learning impairments and motor dysfunctions in adult Lhx5-deficient mice displaying hippocampal disorganization; *Physiol. Behav.* **73** 781-792

Pfaff S, Mendelsohn M, Stewart C, Edlund T and Jessell T 1996 Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation; *Cell* **84** 309-320

Pierani A, Moran-Rivard L, Sunshine M, Littman D, Goulding M and Jessell T 2001 Control of interneuron fate in the developing spinal cord by the progenitor homeodomain protein Dbx1; *Neuron* **29** 367-384

Pierce-Shimomura J, Faumont S, Gaston M, Pearson B and Lockery S 2001 The homeobox gene lim-6 is required for distinct chemosensory representations in *C. elegans; Nature* **410** 694-698

Porter F, Drago J, Xu Y, Cheema S, Wassif C, Huang S, Lee E, Grinberg A, Massalas J, Bodine D, Alt F and Westphal H 1997 Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development; *Development* **124** 2935-2944

Pressman C, Chen H and Johnson R 2000 LMX1B, a LIM homeodomain class transcription factor, is necessary for normal development of multiple tissues in the anterior segment of the murine eye; *Genesis* **26** 15-25

Pueyo J, Galindo M, Bishop S and Couso J 2000 Proximal-distal leg development in *Drosophila* requires the apterous gene and the Lim1 homologue dlim1; *Development* **127** 5391-5402

Raetzman L, Ward R and Camper S 2002 Lhx4 and Prop1 are required for cell survival and expansion of the pituitary primordia; *Development* **129** 4229-4239

Rauskolb C, Correia T and Irvine K 1999 Fringe-dependent separation of dorsal and ventral cells in the *Drosophila* wing; *Nature* **401** 476-480

Retaux S, Rogard M, Bach I, Failli V and Besson M 1999 Lhx9: a novel LIM-homeodomain gene expressed in the developing forebrain; *J Neurosci.* **19** 783-793

Rincon-Limas D, Lu C, Canal I, Calleja M, Rodriguez-Esteban C, Izpisua-Belmonte J and Botas J 1999 Conservation of the expression and function of apterous orthologs in *Drosophila* and mammals; *Proc. Natl. Acad. Sci. U S A* **96** 2165-2170

_____, Lu C, Canal I and Botas J 2000 The level of DLDB/CHIP controls the activity of the LIM homeodomain protein apterous: evidence for a functional tetramer complex *in vivo; EMBO J.* **19** 2602-2614

Ringo J, Werczberger R, Altaratz M, and Segal D 1991 Female sexual receptivity is defective in juvenile hormone-deficient mutants of the apterous gene of *Drosophila melanogaster*; *Behav. Genet.* 21 453-469

_____, _____ and Segal D 1992 Male sexual signaling is defective in mutants of the apterous gene of *Drosophila melanogaster*; *Behav. Genet.* **22** 469-487

Rodriguez-Esteban C, Schwabe J, Pena J, Rincon-Limas D, Magallon J, Botas J and Belmonte J 1998 Lhx2, a vertebrate homologue of apterous, regulates vertebrate limb outgrowth; *Development* **125** 3925-3934

Roelink H, Porter J, Chiang C, Tanabe Y, Chang D, Beachy P and Jessell T 1995 Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis; *Cell* **81** 445-455

Rohr C, Prestel J, Heidet L, Hosser H, Kriz W, Johnson R, Antignac C and Witzgall R 2002 The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes; *J. Clin. Invest.* **109** 1073-1082

Sagasti A, Hobert O, Troemel E, Ruvkun G and Bargmann C 1999 Alternative olfactory neuron fates are specified by the LIM homeobox gene lim-4; *Genes. Dev.* **13** 1794-1806

Sanchez-Garcia I, Osada H, Forster A and Rabbitts T1993 The cysteine-rich LIM domains inhibit DNA binding by the associated homeodomain in Isl-1; *EMBO J.* **12** 4243-4250

Sarafi-Reinach T, Melkman T, Hobert O and Sengupta P 2001 The lin-11 LIM homeobox gene specifies olfactory and chemosensory neuron fates in C. elegans; *Development* **128** 3269-3281

Schneider L, O'Brien M and Taghert P 1991 *In situ* hybridization analysis of the FMRFamide neuropeptide gene in *Drosophila*. I. Restricted expression in embryonic and larval stages; *J. Comp. Neurol.* **304** 608-622

Sharma K, Sheng H, Lettieri K, Li H, Karavanov A, Potter S, Westphal H and Pfaff S 1998 LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons; *Cell* **95** 817-828

_____, Leonard A, Lettieri K and Pfaff S 2000 Genetic and epigenetic mechanisms contribute to motor neuron pathfinding; *Nature* **406** 515-519

Shawlot W and Behringer R 1995 Requirement for Lim1 in head-organizer function; *Nature* **374** 425-430

_____, Wakamiya M, Kwan K, Kania A, Jessell T and Behringer R 1999 Lim1 is required in both primitive streak-derived tissues and visceral endoderm for head formation in the mouse; *Development* **126** 4925-4932

Sheng H, Zhadanov A, Mosinger B J, Fujii T, Bertuzzi S, Grinberg A, Lee E, Huang S, Mahon K and Westphal H 1996 Specification of pituitary cell lineages by the LIM homeobox gene Lhx3; *Science* **272** 1004-1007

_____, Bertuzzi S, Chiang C, Shawlot W, Taira M, Dawid I and Westphal H 1997 Expression of murine Lhx5 suggests a role in specifying the forebrain; *Developmental Dynamics* **208** 266-277

Shibaguchi T, Kato J, Abe M, Tamamura Y, Tabata M, Liu J, Iwamoto M, Wakisaka S, Wanaka A and Kurisu K 2003 Expression and role of Lhx8 in murine tooth development; *Arch. Histol. Cytol.* **66** 95-108

Shirasaki R and Pfaff S 2002 Transcriptional codes and the control of neuronal identity; *Annu. Rev. Neurosci.* **25** 251-281

Smidt M, Asbreuk C, Cox J, Chen H, Johnson L and Burbach J 2000 A second independent pathway for development of mesencephalic dopaminergic neurons requires Lmx1b; *Nat. Neurosci.* **3** 337-341

Sockanathan S 2003 Towards cracking the code: LIM protein complexes in the spinal cord; *Trends Neurosci.* **26** 57-59

Stevens M and Bryant P 1985 Apparent genetic complexity generated by developmental thresholds: the apterous locus in *Drosophila melanogaster; Genetics* **110** 285-297

Tanabe Y and Jessell T 1996 Diversity and pattern in the developing spinal cord; *Science* **274** 1115-1123

Tanabe Y, William C and Jessell T 1998 Specification of motor neuron identity by the MNR2 homeodomain protein; *Cell* **95** 67-80

Thaler J, Lee S, Jurata L, Gill G and Pfaff S 2002 LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions; *Cell* **110** 237-249

Thor S, Ericson J, Brannstrom T and Edlund T 1991 The homeodomain LIM protein Isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat; *Neuron* **7** 881-889

_____ and Thomas J 1997 The *Drosophila* islet gene governs axon pathfinding and neurotransmitter identity; *Neuron* **18** 397-409

_____, Andersson S, Tomlinson A and Thomas J 1999 A LIM-homeodomain combinatorial code for motor-neuron pathway selection; *Nature* **397** 76-80

_____, Ragsdale C and Grove E 2000 Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J); *Dev. Biol.* **217** 254-265

Torigoi E, Bennani-Baiti I, Rosen C, Gonzalez K, Morcillo P, Ptashne M and Dorsett D 2000 Chip interacts with diverse homeodomain proteins and potentiates bicoid activity *in vivo; Proc. Natl. Acad. Sci. U S A* **97** 2686-2691

Tsang T, Shawlot W, Kinder S, Kobayashi A, Kwan K, Schughart K, Kania A, Jessell T, Behringer R and Tam P 2000 Lim1 activity is required for intermediate mesoderm differentiation in the mouse embryo; *Dev. Biol.* **223** 77-90

Tsuchida T, Ensini M, Morton S, Baldassare M, Edlund T, Jessell T and Pfaff S 1994 Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes; *Cell* **79** 957-970

Tsuji T, Sato A, Hiratani I, Taira M, Saigo K and Kojima T 2000 Requirements of Lim1, a *Drosophila* LIM-homeobox gene, for normal leg and antennal development; *Development* **127** 4315-4323

Valge-Archer V, Osada H, Warren A, Forster A, Li J, Baer R and Rabbitts T 1994 The LIM protein RBTN2 and the basic helix-loop-helix protein TAL1 are present in a complex in erythroid cells; *Proc. Natl. Acad. Sci. U S A* **96** 8617-8621

van Meyel D, O'Keefe D, Jurata L, Thor S, Gill G and Thomas J 1999 Chip and apterous physically interact to form a functional complex during *Drosophila* development; *Mol. Cell.* **4** 259-265

van Meyel D, O'Keefe D, Thor S, Jurata L, Gill G and Thomas J 2000 Chip is an essential cofactor for apterous in the regulation of axon guidance in *Drosophila; Development* **127** 1823-1831

_____, Thomas J and Agulnick A 2003 Ssdp proteins bind to LIM-interacting co-factors and regulate the activity of LIM-homeodomain protein complexes *in vivo; Development* **130** 1915-1925

Varela-Echavarria A, Tucker A, Puschel A and Guthrie S 1997 Motor axon subpopulations respond differentially to the chemorepellents netrin-1 and semaphorin D; *Neuron* **18** 193-207

Wadman I, Li J, Bash R, Forster A, Osada H, Rabbitts T and Baer R 1994 Specific in vivo association between the bHLH and LIM proteins implicated in human T cell leukemia; *EMBO J.* **13** 4831-4839

Wang S, Simcox A and Campbell G 2000 Dual role for *Drosophila* epidermal growth factor receptor signaling in early wing disc development; *Genes. Dev.* **14** 2271-2276

Way J and Chalfie M 1988 mec-3, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in *C. elegans; Cell* **54** 5-16

Weihe U, Milan M and Cohen S 2001 Regulation of Apterous activity in *Drosophila* wing development; *Development* **122** 4615-4622

Wijgerde M, McMahon J, Rule M and McMahon A 2002 A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord; *Genes. Dev.* **16** 2849-2864

Zecca M and Struhl G 2002a Control of growth and patterning of the *Drosophila* wing imaginal disc by EGFR-mediated signaling; *Development* **129** 1369-1376

_____ and _____ 2002b Subdivision of the *Drosophila* wing imaginal disc by EGFR-mediated signaling; *Development* **129** 1357-1368

Zhao Y, Sheng H, Amini R, Grinberg A, Lee E, Huang S, Taira M and Westphal H 1999 Control of hippocampal morphogenesis and neuronal differentiation by the LIM homeobox gene Lhx5; *Science* **284** 1155-1158

Zhu T, Bodem J, Keppel E, Paro R and Royer-Pokora B 1995 A single ancestral gene of the human LIM domain oncogene family LMO in *Drosophila:* characterization of the *Drosophila* Dlmo gene; *Oncogene* **11** 1283-1290