Common Molecular Pathways for Patterning of the Body Axis, Limbs, Central Nervous System, and Face during Embryonic Development

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Many congenital anomalies affecting the face are known to appear as syndromes or associations, in combination with other defects. Often, these involve the limbs, eyes, central nervous system, and body axis. A general, and understandable, tendency among clinical researchers has been to seek a single cell type or definable embryologic process on which to ascribe the etiologic basis for such associations. The possibility of a gene, or group of genes, under coordinate control has not received much attention until recently. With the advent of recombinant DNA technology and the current explosion in basic research on the molecular bases of embryonic development, however, several possibilities are beginning to emerge. Here, I will list a few genes whose expression during development suggests that the molecules they encode are used as part of a coordinate molecular pathway, and that they play a role in the development of systems that often appear together in congenital associations or syndromes.

KEY WORDS: *congenital anomalies, gene groups, molecular biology*

Hundreds of molecules are already known to play a role in specific developmental processes. The few that are considered here have been selected because of the similarity of their spatial or temporal patterns of expression in the different systems referred to in the title. Some of these molecules are secreted peptides, others are receptors for these peptides or for retinoic acid (which appears to play a role in craniofacial, limb, and body axis development), and a final class comprises transcription factors, which regulate the expression of other genes.

Peptide Growth Factors and Their Receptors

The TGFβ superfamily is a large family of molecules which includes several members with apparent roles in the development of the body axis, limbs, and face. Among them are the **activins and inhibins**. The former have been shown to act as mesodermal inducers during very early embryonic development. Recently, activin-βA has been shown to be expressed strongly in craniofacial mesenchyme (Feijen et al., 1994). In addition, two **serine-threonine kinase receptors**, which bind activin and related peptides, are also expressed in restricted domains in these systems. The IIA receptor (also known as Type-II receptor) is expressed early in development in the primitive streak and Hensen's node with strong left-right asymmetry and later, in the notochord and myotomal compartment of the somites. In the limb, it is expressed in the precartilage condensations that precede bone formation; in the nervous system it is seen in hindbrain rhombomeres and in the forebrain. In the head, the IIA receptor is expressed most strongly in the third arch (Stern et al., 1995). A related receptor, terms IIB, is also expressed initially in the primitive streak but is symmetric; later expression in the limb is restricted to the proximal part on the radial aspect of the limb bud, the dorsal neural tube and hindbrain rhombomeres, and restricted regions of the forebrain. In the face, it is expressed most strongly in the third arch and frontonasal process (Stern et al., 1995).

Other members of the TGFβ superfamily of growth factors, the TGFβs themselves, and the bone morphogenetic proteins (BMPs) are also expressed in all these systems. For example, TGFβ3 is expressed in precartilage condensations in the limb and in the midline of the palatal mesenchyme (Ferguson, 1995; Gehris et al., 1994). Some of the BMPs are expressed in the primitive streak during gastrulation. Later, BMP-2, -2A, -4, -6, and -7 show various patterns of regional expression in the limbs and BMP-2, -4, and -7 are expressed in the dorsal neural tube and ectoderm; several members are expressed in craniofacial mesenchyme, tooth buds, and other structures in the face (Lyons et al., 1990).

Other families of secreted growth factors also exist, including the ones containing hepatocyte growth factor or scatter factor (HGF/SF) (containing at least two members with developmental expression and role), Wnt (about 13 members), Sonic hedgehog (Shh) (three members) and fibroblast growth factor (FGF) (eight members). Of these families, the former three are known to have restricted patterns of expression and defined roles in development of the body axis, limbs, nervous system, and face. The latter is known to be involved in development of the body axis and limbs, but a role in craniofacial development has not yet been demonstrated clearly.

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HGF/SF is expressed first in Hensen's node, where it plays a role in the early stages of neural induction (Streit et al., 1995). Later it is expressed in the somites and neural tube (particularly in the forebrain and boundaries between hindbrain rhombomeres), and is a predictor of the position at which the limb buds will start to elevate long before these become visible (Théry et al., 1995). In the face, HGF/SF is expressed in the mesenchyme of all the branchial arches, in the pharyngeal endoderm and in the cranial neural crest during its migration. Its receptor is **c-met**, a transmembrane **tyrosine kinase**, which is ubiquitously expressed during gastrulation, and is then concentrated in limb mesenchyme, spinal nerves, motor columns, and dorsal root ganglia. It is also expressed in the dorsomedial edge of each somite, from which the myotomes start to form. In the prospective head region, c-met is expressed most strongly in the first four (occipital) somites, which will contribute to the muscle of the tongue and occipital muscles, and in the pharyngeal endoderm and endothelial lining of aortic arches (Théry et al., 1995).

The family of secreted growth factors known as **Wnt**, some of whose members are believed to be involved in modulating mesoderm induction and axial patterning, also displays restricted patterns of expression within the limb and nervous system. In the face, several members of this family also have restricted patterns of expression in the branchial arches (Augustine et al., 1993; Ku and Melton, 1993).

A newly discovered secreted peptide morphogen is Sonic hedgehog (Shh), which starts to be expressed in Hensen's node and notochord during neurulation, from where expression spreads to the overlying floor plate of the developing neural tube, the zona limitans in the diencephalon, the infundibulum, and other restricted regions of the forebrain. In the limb, it is concentrated at the ulnar side of the early limb bud. Shh protein has been shown to be able to induce a floor plate and motor axons from the neural tube, as well as to polarize the limb if placed ectopically, on the radial side (Johnson et al., 1994; Pownall, 1994). In the head, Shh is expressed most strongly on the maxillary-facing side of the mandible arch, the tongue, palate, third arch, frontonasal process, and around the nasal pits (Stern, unpublished observations).

Transcription Factors

Transcription factors have also been classified into several families by structural and functional criteria. One of these families is characterized by the homeobox, a 184-base pair-long sequence of DNA that is highly conserved in many different organisms and which binds specific DNA sequences. Some of the homeobox containing genes (called Hox genes) are organized into four clusters (a total of 40 genes), with defined spatial and temporal expression in all the systems being discussed here. Indeed, it has been suggested that the developmental pathways followed by each branchial arch is encoded in the specific combination of Hox genes expressed by each arch, as a **branchial Hox-code** (Hunt et al., 1991; Krumlauf, 1993).

Other genes containing a homeobox do not fall into clusters. Among them are MHox, engrailed and goosecoid. **MHox** is initially expressed in the lateral plate mesoderm, dermatome, and dermis. Later, it appears in limb bud mesenchyme and in branchial epithelium and mesenchyme, where the epithelium has been shown to be required for the maintenance of expression in the underlying mesenchyme (Kuratani et al., 1994). The **engrailed** proteins (two have been described) are expressed at the midbrain-hindbrain junction (presumptive cerebellum) and in the optic rectum. One of them (En-1) is restricted dorsoventrally in the limb buds. Engrailed is also expressed in the mandible and in the anterior pituitary (Hemmati-Brivanlou et al., 1991; Gardner and Barald, 1992). **Goosecoid** differs from the homeobox genes mentioned above in that it possesses a lysine residue in position 50 of the homeobox, which alters its binding specificity to DNA. It is first expressed in Hensen's node and its precursor cells and in the prechordal plate and its descendant cells (which give rise to the extrinsic muscles of the eye) (Izpisúa-Belmonte et al., 1993). In the limb, its expression is restricted proximally and to the radial aspect; in the forebrain it is found as a gradient, increasing from basal to dorsal. In the face, its expression is strongest in the first branchial arch and in the palatal shelves during fusion (Gaunt et al., 1993; Stern, unpublished observations).

Transcription factors without a homeobox, which are also expressed in interesting patterns in the body axis, nervous system, limbs, and face include AP-2, the distalless-related family (Dlx) and gli. **AP-2** is expressed in many neural crest derived cells (including branchial arch mesenchyme), limb bud mesenchyme, a longitudinal column in the spinal cord and hindbrain, cranial and sensory ganglia, and kidney (meso- and metanephros) (Mitchell et al., 1991). **Dlx** family members are expressed in skeletal elements, basal telencephalon, ventral diencephalon, cranial ganglia, facial ectoderm, and branchial arches (Bulfone et al., 1993; Dirksen et al., 1993; Simeone et al., 1994). Finally, **gli**, a zinc-finger transcription factor, is expressed in digital mesenchyme condensations in the limb, the ependymal layer of the spinal cord, the occipital region, and Meckel's cartilage (Walterhouse et al., 1993).

Conclusion

The above list, though extensive, is not exhaustive and the patterns of expression of these genes have been simplified considerably for brevity. It should, however, give an idea of the emerging complexity of such patterns of expression and the sheer number of genes, already known to play developmentally important roles, whose expression suggests involvement in many apparently diverse developmental processes, which appear otherwise to have little in common. In the 1930s, C.H. Waddington and other embryologists were strong proponents of the idea of developmental fields. A field is defined as "a portion of an embryo contained within well-defined boundaries, which can develop independently, without instructive influences from the rest of the embryo." Initially, the whole embryo is a single field, but during development it subdivides