



The Story of the Hand

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Abstract

This review describes the Story of the Human Hand. It traces the functional needs that led to evolution of the human hand as well as its embryological development. The various in utero stages of formation of the human hand are covered along with a description of the various molecular and genetic factors that control this process.

Keywords

- ▶ embryology
- ▶ hand
- ▶ upper extremity
- ▶ development
- ▶ limb bud

Introduction

“*Ontogeny Recapitulates Phylogeny*”; so said Ernst Haeckel in 1866.¹ He was referring to his observation that an embryo passing through various stages of development seemed to reflect the evolutionary path that a particular organism had taken. Earlier stages of embryonic development showed marked similarity across species and even genera, emphasizing the fact that each subsequent evolutionary step was an advancement on the previous one. It is hence important to understand evolution to appreciate embryology (▶ **Fig. 1**).

Development of the human hand is a complex and fascinating process that represents the *pinnacle* of evolution of functional appendages across all living organisms. Thus, to understand the embryological development of the human hand, one needs to begin by asking the obvious question, “*Why do we need hands?*”

Why Do We Need Hands?

Vertebrates developed hands over the course of millennia of evolution. This happened as a result of changes in habitat and habits. A major change in habitat occurred when life, which began in the oceans, migrated to land. As a consequence, water dwelling vertebrates such as fish had to develop appropriate appendages for locomotion on land. Thus, fins gradually transformed into limbs. This is exemplified by the

intermediate stage of “fin-limbs” that we see in the mudskipper, a fish that is known to “walk” on land and can even be seen climbing and clinging on to mangrove stems.

Early land dwellers, such as reptiles, used their limbs for very little other than locomotion. Hence, there is hardly any differentiation between the fore and hindlimbs (▶ **Fig. 2**). The limited need for these appendages led some reptiles such as the skink to reduce their limbs to mere nubbins, while others abandoned them entirely. We know them as snakes!

Later land vertebrates such as birds developed a more specialized use for their limbs. They adapted a different mode of locomotion—flying—and hence the forelimbs transformed into wings. This differentiation evolved even further as the hindlimb adapted itself to walking on land, grasping twigs while perching or even allowing for some limited manipulative skills such as holding food and bringing it to the mouth (▶ **Fig. 3**). This can be deduced by the intermediate stage seen in the chicks of a strange bird of South America known as the hoatzin, whose chicks have claws in their wings that they use to grasp twigs while moving about in the trees.

The next major evolutionary change occurred when mammals developed. The forelimbs became far more specialized and, in addition to locomotion, were used for a variety of functions such as killing prey, digging, scratching, and manipulating objects. Even among mammals, a wide variation in manipulative skills is provided for significant survival advantages of one species over another (▶ **Figs. 4 and 5**).

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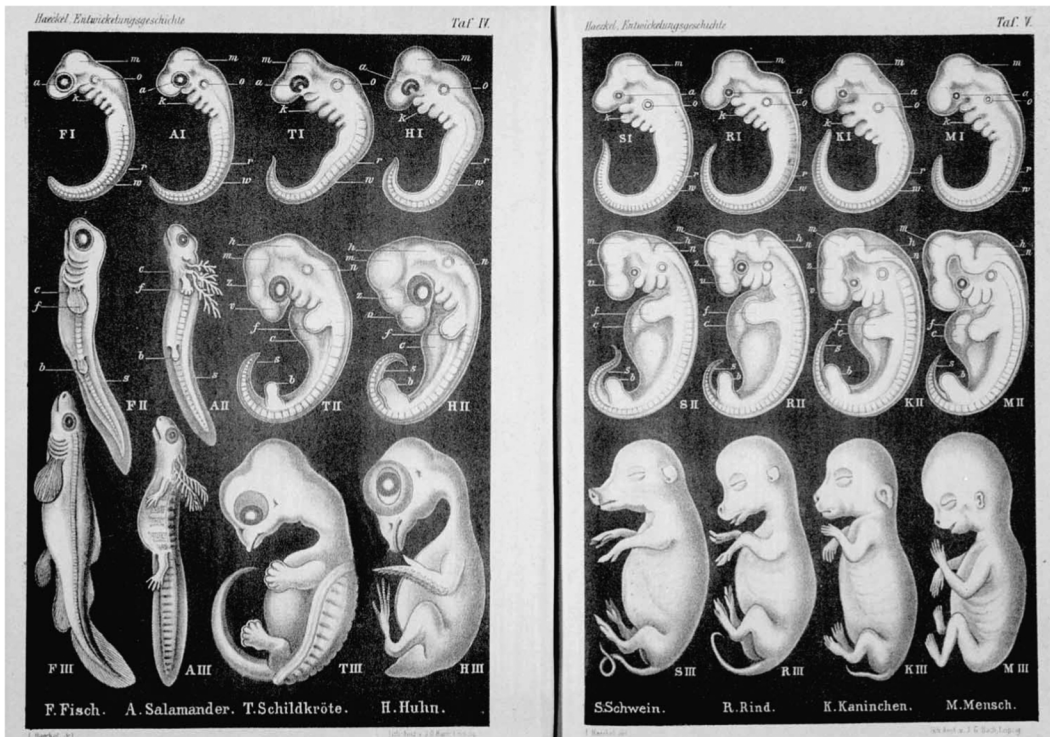


Fig. 1 Ernst Haeckel’s drawings illustrate a stark similarity in the early embryonic stages of vertebrates from fish to humans (Courtesy of Kleinert-Kutz Hand Care Center).



Fig. 2 The fore and hind limbs of reptiles such as this Mexican lizard show significant similarities, suggesting limited manipulative skills (Courtesy of Kleinert-Kutz Hand Care Center).



Fig. 3 This American kestrel, a small falcon, is demonstrating remarkable manipulative skills with its hind limbs. The bird is perched literally at the tip of a tree with one limb, while using the other to bring food to its mouth! (Courtesy of Kleinert-Kutz Hand Care Center).

Over time, as mammals evolved, a minor change in habitat occurred when ground dwellers became tree dwellers. These were the primates, whose mobility depended on their ability to clamber among the branches. This needs to move through trees led to two differentiating functional needs: stereoscopic vision and the ability to grasp. Forward facing eyes allowed stereoscopic vision, while the ability to grasp was achieved by developing opposable thumbs and replacing claws with flat nails. Gradually, as primates evolved, a minor change in habitat was seen. Tree dwellers descended from their lofty perches and became land dwellers. These were the apes!

Apes represent the apex of animal evolution. As they became increasingly bipedal, the forelimbs were left free, allowing for increasing dexterity. Chimpanzees have

repeatedly been seen taking a twig, stripping it of leaves and poking it into termite nests. Warrior termites perceive this twig as an enemy and attack it by biting and grasping onto it. The chimpanzee then withdraws the stick—which is now full of termites—and proceeds to enjoy a hearty insect meal. This ability to not just use, but purposefully create a tool, is unique to apes. We are well aware of the ability of humans to fashion tools all the way from crude stone implements to the sophisticated computer controlled machines of today.

The human hand represents the pinnacle of evolution of all appendages. It not only serves as a motor and sensory organ but also has the ability of *stereognosis*. It is this last



Fig. 4 The deer, which is an ungulate, essentially lacks digits. Thus, it has no manipulative skills and relies entirely on its mouth to feed itself (Courtesy of Kleinert-Kutz Hand Care Center).



Fig. 5 In stark contrast to the deer, this squirrel clearly demonstrates superior manipulative skills because of the presence of fingers (Courtesy of Kleinert-Kutz Hand Care Center).

quality that enables us to put our hands in our pockets or purse and identify—merely by touch—the various objects therein. And if that itself weren't enough, the human hand has an ultimate role; it serves as an *organ of expression*! We all are used to seeing people use their hands to gesticulate while speaking. This is uniquely human!

Sequence of Limb Development

For all its complexity, formation of the forelimb takes a mere 30 days from start to finish!

Limb development commences around day 26 of embryonic life.² The entire sequence can be broadly divided into two phases. *Phase 1* determines the site in the human embryo where the limb needs to develop. This results in the positioning of *limb competent tissue*, whereby totipotent ectodermal and mesodermal elements get specialized and are directed toward the formation of a limb. Consequently, a *limb bud* arises from the somatic lateral plate mesoderm. It consists of a central mesodermal core with an ectodermal envelope. The bud extends laterally from the embryo and is dorsoventrally

flattened (► **Fig. 6**). *Phase 2* consists of the actual growth and patterning of this limb bud.

By day 33, a tripartite skeleton is formed, consisting of the *stylopod*, *zeugopod*, and *autopod* (► **Fig. 7**).² The stylopod accounts for the region that will eventually form the shoulder girdle and humerus. The zeugopod is the forearm region with radius and ulna, while the autopod consists of the *hand plate* wherein the carpus and digital rays are formed.

Limb growth occurs along *three axes*: proximodistal, dorsoventral, and radioulnar. It is important to know that while speaking of embryonic growth, the term radioulnar is sometimes interchangeably used with anteroposterior or pre and postaxial. Growth and differentiation along these three axes are respectively directed by *three control regions*: progress zone (PZ), apical ectodermal ridge (AER), and zone of polarizing activity (ZPA) (► **Figs. 8 and 9**).^{2,3}

Limb growth is a three-dimensional event that occurs as a continuum. All aspects of growth and differentiation occur in all three axes and hence it is impossible to clearly demarcate which control region is responsible for which axis. Every part of the developing limb bud is to varying extent affected by each control region. The PZ is a mesodermal cell collection at the end of the limb bud. It demonstrates robust proliferative activity and is largely responsible for the proximodistal (longitudinal) growth of the limb. However, while the extremity is largely mesodermal and hence dependent on the PZ for actual cell proliferation and growth, it is the AER that secretes the initial molecules that trigger and maintain growth in the PZ.^{3,4} The AER is an ectodermal thickening at the very tip of the limb bud. It is located at the dorsoventral boundary and runs craniocaudally. This ridge is largely responsible for the differentiation of the flexor and extensor sides of the extremity.^{5,6} The ZPA is also a mesodermal cell collection. It is located

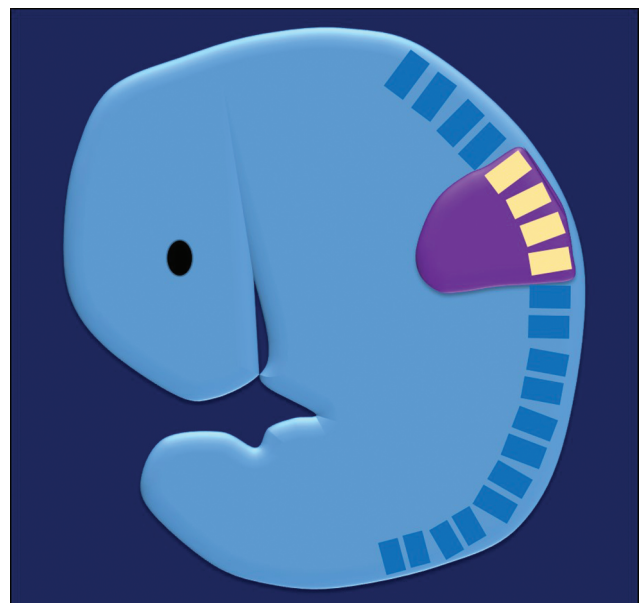


Fig. 6 Mesodermal somatic plates are shown along the length of this embryo. Those designated for the limb are in yellow and the developing limb bud is shown in purple (Courtesy of Kleinert-Kutz Hand Care Center).

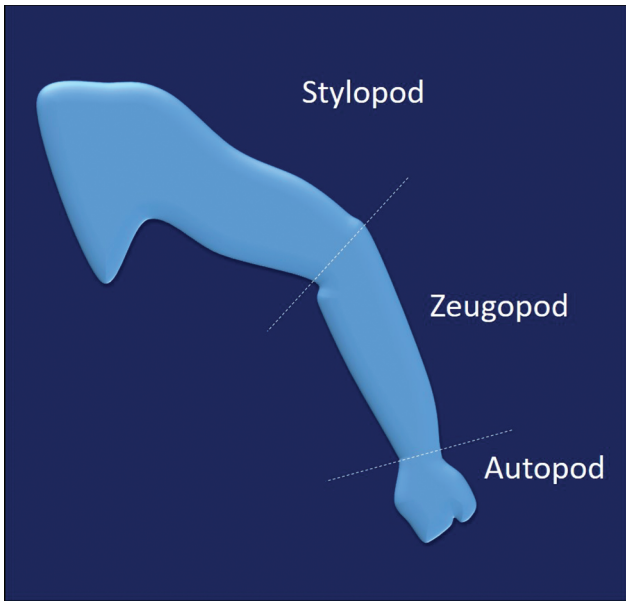


Fig. 7 The stylopod forms the shoulder girdle and humerus; zeugopod forms the radius and ulna while the autopod forms the carpus and digital rays (Courtesy of Kleinert-Kutz Hand Care Center).

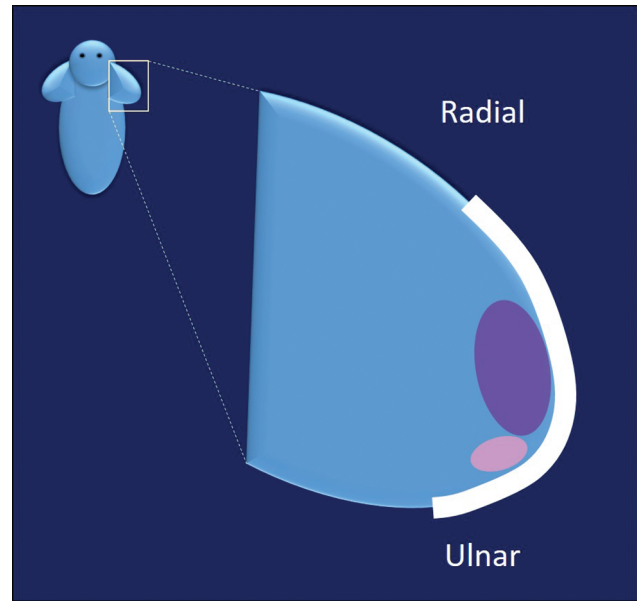


Fig. 8 Frontal view of the limb bud as a dorsoventrally flattened outgrowth showing the apical ectodermal ridge in white, the progress zone in purple, and zone of polarizing activity in pink (Courtesy of Kleinert-Kutz Hand Care Center).

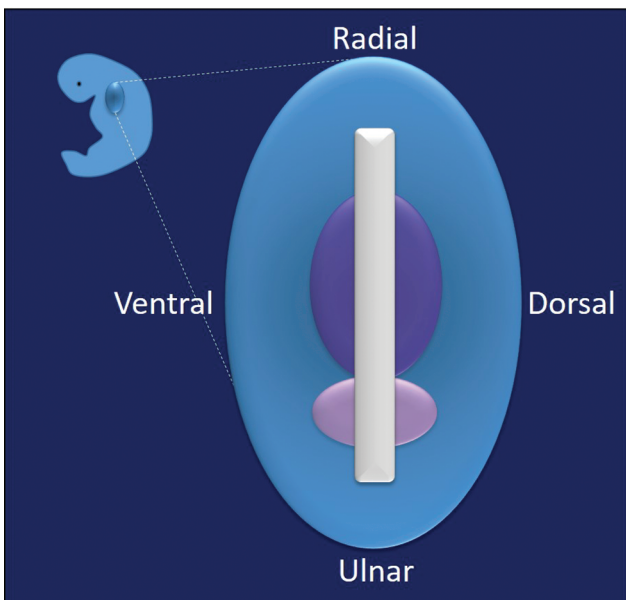


Fig. 9 End-on view of the limb bud as a dorsoventrally flattened outgrowth showing the apical ectodermal ridge in white, the progress zone in purple, and zone of polarizing activity in pink (Courtesy of Kleinert-Kutz Hand Care Center).

ulnarly (posterior or postaxial) at the tip of the growing bud and directs radioulnar patterning.⁷

The entire process of limb development occurs in the second month of gestation. As mentioned earlier, limb bud formation commences around day 26. The tripartite skeleton and hand plate are seen by day 33. Joints develop and flexion at the level of the elbow occurs by day 51. All major features are seen by day 56. After that, most limb development involves increase in size with very little additional differentiation.

There are two overlapping theories that explain the growth and maturation of the limb bud. The first theory suggests that a constant interaction among controlling molecules determines limb development. As limb growth progresses in the proximodistal direction, the PZ advances.⁸ The mesoderm that falls out of the PZ undergoes maturation forming structures such as bones, muscles, tendons, and joints.^{8,9} The second and more widely accepted theory suggests that the genes—specifically *Hox* genes—are responsible for determining the spatial and temporal orientation of the limb. So along with determining the position of the limb bud, these genes also determine its development into a tripartite skeleton, each part being controlled by specific *Hox* genes.^{10,11}

Molecular Control of Limb Development

Limb formation is controlled for the most part by a few classes of molecules. These are part of major signal pathways and initiate a *developmental cascade*. Therefore, fewer molecules are involved in the initial phases of development but trigger a growing cascade of molecules that in turn orchestrate a response from hundreds of other molecules. This cascade mechanism bears remembering because any problem affecting the initial control molecules can have devastating consequences on the entire limb, while problems affecting later molecules may be more confined in their effect.

All these molecular factors exert their influence via major signal pathways, important among which are Wnt family, transforming growth factor-beta (bone morphogenic protein [BMP] being one of them), fibroblast growth factor (FGF), retinoid nuclear receptor, Sonic Hedge-Hog (Shh), notch delta factor, etc.^{2,12}

Molecular control factors belong mostly to four categories: *transcription factors, direct regulators, indirect regulators, and*

modifying factors (►Fig. 10).² Transcription factors interact directly with specific DNA sequences or genes. A single transcription factor may regulate hundreds of genes by either “up” or “down” regulating them. Direct regulators are hydrophobic molecules that pass through the cell membrane and react with nuclear receptors. This in turn may activate or suppress specific transcription factors. Indirect regulators, on the other hand, act via cell surface receptors. Such surface receptors when activated can initiate intracellular signal transduction that in turn can activate or suppress specific transcription factors. Modifying factors control various components of transcription regulation pathways. They can modify *signal transduction* by increasing or decreasing surface receptors by competitive antagonism. Modifying factors can also control *gene transcription* by altering the affinity of transcription factors to specific gene sequences.

Genetic Control of Limb Development

Transcription factors located on specific genes determine the formation and development of the upper extremity. *Homeodomain Box genes (Hox genes)* are primarily responsible for *homeosis* or differentiation of various parts of the body. In humans and other vertebrates, the Hox family consists of 39 genes that occur in four *clusters* (A, B, C, and D) on four different chromosomes. Hox A cluster is located on chromosome 2, Hox B on chromosome 17, Hox C on chromosome 12 and Hox D on chromosome 1. In these clusters, *paralogs* 5, 6, 7, and 8 contain the 11 genes that initially define forelimb formation and differentiation.^{2,13}

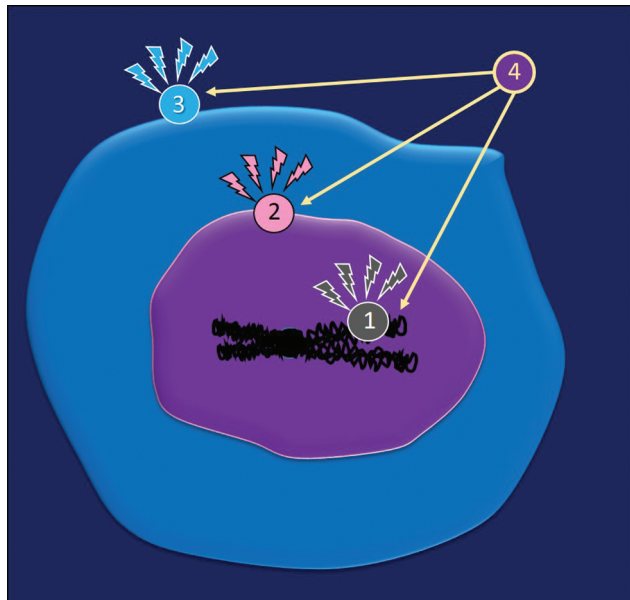


Fig. 10 Schematic representation of a cell with the cell membrane in blue, nucleus in purple, and chromosome in black. The four categories of molecular controls are shown at their site of action: transcription factors (#1 acting on DNA), direct regulators (#2 acting on the nucleus), indirect regulators (#3 acting on the cell membrane), and modifying factors (#4 acting on and modifying all the other three factors) (Courtesy of Kleinert-Kutz Hand Care Center).

Sequence of Limb Growth

As mentioned earlier, the sequence of limb development can be divided into two phases. *Phase 1* determines the site in the human embryo where the limb needs to develop and *Phase 2* consists of the actual growth and patterning of this limb bud. Two critical events occur in *Phase 1*: the first specifies limb boundaries under the control of Hox transcription factors, while the second critical event heralds the appearance of polarizing activity under the influence of Hox B, Tbx 2, Cux 2, and d-Hand genes.^{14,15} *Phase 2* of limb development involves proximodistal growth, dorsoventral patterning, and radioulnar differentiation.²

Proximodistal Growth

Initiation of proximodistal growth occurs by the secretion of various molecules in the AER. However, it is important to note that the AER is an ectodermal structure, while all elements of the upper extremity, except skin and nails, are of mesodermal origin. Hence, it is important to realize that actual proximodistal growth occurs in the PZ, which is a zone of mesodermal cell proliferation.¹⁶ Longitudinal growth of the limb bud commences with the induction of Fgf 10 in the limb field mesoderm.^{2,3} This stimulates formation of the PZ, which shifts distally forming the three limb segments.^{16,17} Fgf 10 induces production of Fgf 4, Fgf 8, Fgf 9, and Fgf 17 that maintain the PZ. The stylopod is formed first followed by the zeugopod and finally the autopod.^{2,17} Stylopod formation is principally induced by Hox A-10, zeugopod by Hox A-11 and the autopod by Hox A-13.² Formation of the actual hand occurs in the autopod by focal loss of AER and Fgf occurring at the presumptive web spaces. This induces a BMP-related apoptosis which leads to the formation of digital rays.¹⁸

As limb development progresses, growth differentiation factor 5 demarcates the sites of joint formation. Another set of genes known as SRY-Box (SOX) genes are responsible for the secretion of transcription factors (especially SOX 9) that control cartilage formation. Two separate factors, scleraxis and tendon, induce actual joint formation in the mesoderm. Other molecules such as Pax 3, Six 1, Six 2, Eya 1, and Eya 2 specify formation of muscles, tendons, and ligaments.^{19,20}

Dorsoventral Patterning

This begins in the lateral plate mesoderm that induces the secretion of Wnt in the overlying ectoderm. Wnt, which is initially present everywhere in the limb bud, gets confined to the dorsal ectoderm by BMP-induced activation of the transcription factor engrailed-1 (En-1).¹³ Radical fringe (another transcription factor) is expressed at the boundary between Wnt and En-1 thus demarcating cells destined to form the AER.^{3,6} Under the influence of Wnt, Lmx1b is expressed in the dorsum and is principally responsible for the differentiation of the dorsal (extensor) structures.^{7,21}

Radioulnar Differentiation

This patterning, which eventually leads to the formation of a thumb on the radial side, is also variably known as anteroposterior or pre-post axial differentiation. Two relatively independent mechanisms control this part of limb development: the ZPA and Hox factors 5–13.^{2,22,23} Retinoic acid and Shh play a pivotal role in inducing both these mechanisms.^{2,7} These factors are located within the ZPA and posteriorize (ulnarize) the digits. Ectopically located ZPA in the radial part of the limb bud led to a mirror hand. Digital differentiation in the autopod is influenced by transcription factors Gli and Ci of the Shh pathway.^{2,24} Absence of Gli in a limb bud led to polydactyly with no radioulnar differentiation.²⁴

Formation of the Thumb

Of all digits, nothing defines the uniqueness of a human hand like the pollux. The human thumb epitomizes the pinnacle of evolution of all animal limbs. Not unexpectedly therefore, in line with its exceptional function and identity, the thumb also has an exclusive story of its formation. It is the last digit to form, possibly suggesting that it is the last modification in the developmental sequence determined by *Hox* genes.²⁵ Unfortunately, all information about digital differentiation is obtained via experiments on lower animals, which automatically preclude obtaining a clear picture of the human thumb formation.

Hox genes and molecules of the Shh pathway are both involved in an intricate interplay that leads to radio-ulnar differentiation of digits.²⁵ However, strangely enough, it is the absence of factors/activity rather than a direct proactive set of events that lead to thumb formation. In a paradoxical way, it appears that the thumb forms more by omission rather than commission. *Hox* genes 10–13 are present throughout the autopod. However, Hox 10–12 are suppressed in the area of the future thumb allowing expression only of Hox 13.^{25–27} In an almost parallel manner, Gli3, which is a downstream molecule of the Shh pathway, has a natural tendency to get converted to its repressor variant Gli3r.^{25,28} This tendency is curbed by the Shh pathway in a gradient manner from the posterior (ulnar) to anterior (radial) side.²⁵ Consequently, in a warped, almost double-negative manner, Gli3 remains expressed in the radial side of the autopod, leading to thumb formation.

Embryological Basis of Some Common Hand Conditions

Accumulated knowledge of factors controlling and directing limb growth has allowed a better understanding of the etiology of some common hand conditions. Experimental studies have further permitted manipulation of specific genes or transcription factors in animal models, leading to the creation of limb anomalies that remarkably mimic those seen in humans. The fact that controlling molecules function through a cascade has led us to realize that errors in molecules higher up the cascade can cause widespread anomalies

in the extremity, while problems affecting molecules lower down the cascade result in more localized deformities.

Disruption along the Proximal–Distal Axis (Transverse Arrest)

FGF under influence of AER maintain the PZ and ZPA-related limb outgrowth. Loss or disruption of the AER or loss of FGF receptors leads to truncation of limb and has been demonstrated in experimental models.²⁹ Clinically this manifests itself in various forms of transverse arrest (e.g., *phocomelia*). The level of arrest depends on when disruption occurs since formation of the stylopod, zeugopod, and autopod is both a spatial and temporal event.^{30,31} AER-induced FGFs also influence Wnt pathways and have been implicated in some forms of *brachydactyly*.¹²

Disruption along the Radioulnar Axis (Longitudinal Deficiencies)

Radioulnar differentiation is largely controlled by the AER. Defects in this aspect of limb development can lead to varying degrees of radial or ulnar deficiencies.^{12,32}

While complete AER disruption or loss causes transverse arrest, reduction in AER-induced FGF function has been implicated in the development of longitudinal deficiencies. Limbs with reduced FGF are reduced in length, volume, and width. The natural tendency of the extremity is toward “ulnarization.” This drive toward formation of the ulnar side of the hand persists under influence of ZPA. Formation of radial structures occurs under the influence of FGF. The resulting deficiencies in experimental models with reduced FGFs are very similar to the clinical spectrum that leads to radial longitudinal deficiencies (*radial clubhand*).^{32–34} Deformities in syndromes such as Apert, Pfeiffer, or Saethre-Chotzen are associated with mutations in FGF receptors and also demonstrate radial/anterior joint abnormalities as well as *radio-ulnar synostoses*.¹²

Growth and proliferation of the ulnar side of the limb are under a gradient action of Shh that is controlled by the ZPA. Thus, progressive loss of Shh expression is likely to reduce limb volume and growth on the ulnar (post-axial) side of the developing limb bud.^{35,36} Shh-deficient phenotypes in experimental models have been shown to mimic the spectrum of ulnar longitudinal deficiency (*ulnar clubhand*).³⁶

As discussed earlier, there is considerable overlap in the influence of various controlling regions and molecules. Shh, while primarily responsible for the ulnarization drive of growth, is also involved in maintenance of the Shh-FGF loop. Hence, disruption of Shh activity can also lead to defects in radial structures as well as overall limb growth. This explains the occurrence of *thumb deficiencies* in a child with an ulnar clubhand.¹²

Disruption of Digit Formation and Differentiation

As mentioned earlier, formation of the actual hand occurs in the autopod by focal loss of AER and FGF occurring at the presumptive web spaces. This induces a BMP-related apoptosis that results in the formation of digital rays. A disruption of this orderly event can lead to either failure of separation of

digits (*syndactyly*) or break-up of the autopod into too many digits (*polydactyly*).

Animals that demonstrate the presence of natural interdigital webbing such as ducks or bats are shown to express Gremlin in the autopod. This molecule, which is a natural inhibitor of BMP, limits apoptosis within the autopod leading to the development of syndactylous or webbed digits.^{37,38} In humans, overexpression of such BMP inhibitors in the limb mesenchyme leads to maintenance of FGF function resulting in a failure of separation of digits (*syndactyly*).³⁹ Mutation in Noggin (NOG), another BMP inhibitor, is clinically associated with complex syndactyly and polydactyly.¹²

Apert syndrome involves a slightly different mechanism that leads to anomalous development of the hand. In this syndrome, mutations have been detected in FGF receptors that increase their affinity for FGF ligands and glycosaminoglycans that are diffusely present in the limb mesenchyme. This in turn leads to a continuous stimulation that overcomes the BMP pathway. Consequently, apoptosis fails to occur leading to complex syndactyly or *acrosyndactyly*.⁴⁰

Abnormalities in the Shh-Gli3-Gli3r signaling cascade can lead to polydactyly.^{28,41,42} Overexpression of Gli3 its normal form leads to polydactyly.²⁸ Complete loss of Gli3 expression disrupts thumb formation thus leading to loss of digital identity that results in a *five-fingered hand*.²⁸ Mutations that lead to a failure of Shh regulation can cause abnormal radial expression of Shh leading to a *triphalangeal thumb*.^{43,44}

During digit formation and differentiation, SOX9 is responsible for induction of cartilage elements in the phalanges. Complete absence of SOX9 results in regression of the limbs because cartilage formation is not induced. This can lead to digital anomalies such as *camptomelia* and *brachydactyly*.⁴⁵

Cleft Hand

Cleft hand is a unique anomaly resulting from an imbalance of FGF and BMP activity in the hand plate. While FGF8 is responsible for the induction of the central digits, FGF 4 induces development of the ulnar digits. A failure to maintain the FGF8 drive leads to BMP-retinoic acid-induced apoptosis in the central part of the autopod, resulting in a cleft hand. Ulnar digits that are under the influence of FGF4 are largely unaffected and hence usually normal or minimally affected in cleft hand.^{46,47}

Summary

In summary, limb development begins with an understanding of the evolution of mammalian species that led to the formation of the human hand. This exquisite appendage that lies at the pinnacle of evolution is a motor organ, sensory organ, stereognostic organ, and as a crowning glory, an organ of expression! Limb growth involves the first phase of positioning of limb competent tissue in the embryo, followed by growth and patterning of the limb bud. Limb growth occurs along three axes: proximodistal, dorsoventral, and radioulnar, respectively, directed by three control regions: PZ, AER, and ZPA. The limb bud forms around day 26 of embryonic life and is largely completed by day 56.

While knowledge of the molecular and genetic basis of limb development is a fascinating and complex subject, its practical applications are still limited. Genetic counseling plays a role in determining the risk of occurrence of limb deformities in families with known genetic defects. Since a hand anomaly can serve as a mirror to the body, detection of specific gene anomalies may allow clinicians to uncover lurking syndromic conditions with potentially more serious organic or metabolic defects. Association of a specific anatomical deformity with a genetic anomaly can help in prognostication about the mental ability of the affected child as well as expected longevity. This in itself will not change the actual procedure that the surgeon chooses, but may play a major role in determining whether or not a procedure ought to be performed at all. The future lies wide open with exciting possibilities of intrauterine surgery and perhaps 1 day, even gene manipulation!

Disclaimer

No financial benefits of any form have been or will be received for part or all of this study.

Conflict of Interest

None.

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