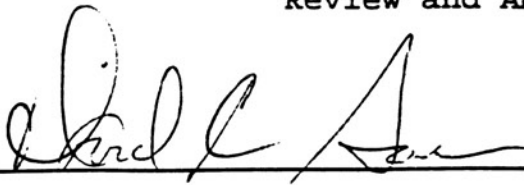
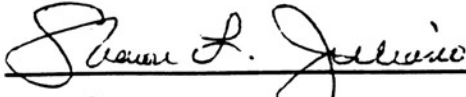


APPROVAL SHEET

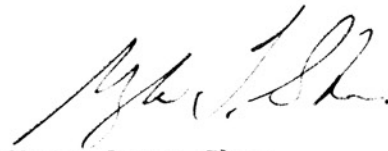
Title of Review: Pattern Formation in Vertebrate Limbs

Name of Candidate: Mary Anne Shea
Master of Science, 1995

Review and Abstract Approved

	(Date) 5/8/95
	(Date) 5/8/95
	(Date) 5/8/95

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A handwritten signature in black ink, appearing to read 'Mary Anne Shea', is positioned above the printed name.

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ABSTRACT

Title of Review: Pattern Formation in Vertebrate Limbs

Mary Anne Shea, Master of Science, 1995

Reviewed directed by: Dr. David C. Beebe, Chairman of Anatomy,
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Patterns are an essential element of all life forms. The concepts of pattern formation are positional information, a mechanism for conductance of the positional information, and an interpretation system. The developmental patterns of the embryonic chick demonstrate two types of patterns. The shape of a bone demonstrates patterns as forms. The processes of development are illustrations of dynamic patterns. In the embryonic chick limb bud, the apical ectodermal ridge exhibits a pattern of required presence for development of the musculature, cartilage elements, vascular system and expression of the homeobox genes. The two current models of pattern formation are the zone of polarizing activity/progress zone model, and the polar coordinate model are reviewed. A new pattern formation model is proposed. The information and memory model is founded on the premise that cells have a working memory which is cumulative, accessible, and passes intact from one cell to its progeny.

Pattern Formation
in Vertebrate Limbs

by
Mary Anne Shea

Thesis submitted to the Faculty of the Department of Anatomy
Graduate Program of the Uniformed Services University of the
Health Sciences in partial fulfillment of the
requirements for the degree of
Master of Science 1995

ACKNOWLEDGEMENTS

I am grateful to Drs. David C. Beebe and Michael Sheridan for their advice and support.

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INTRODUCTION

Patterns are an essential component in all life forms, from the precise molecular pattern in the plasma membrane of a single cell organism to the complex patterns of cells found in the kidney or brain. Understanding how the patterns are formed is crucial to the understanding of the developmental processes.

Early scientists and non-scientists have been observing the patterns of life forms since the time of Aristotle. The developmental biologists in the early 1900's systematically investigated the patterns formed as zygotes of various amphibians or mollusks progressed through the readily observable intermediate stages to adulthood. They understood the zygote contained all the information necessary for development into an adult form. To determine the format of this information, Roux killed, but did not detach, one cell after the initial cleavage of a frog zygote. The remaining cell developed into only half a frog. These results suggested to Roux that the information to form specific tissues was segregated in one or another cell in the first few cleavages of the zygote, as in a mosaic pattern. Destruction of one of these cells he believed, caused the loss of the genes necessary for complete development (Loomis, 1986). Fascinated by Roux's experiments, Hans Dreisch repeated the experiments with several modifications.

Dreisch separated the first two cells of sea urchins by agitation, and the development of the surviving cells was

followed. He was amazed to find a smaller, but complete blastula developed, not half of a blastula, as found by Roux. Dreisch repeated these experiments at the 4 and 8 cell stages, with varying results. At each stage of development, some cells became whole organisms, while others were deformed or died. He decided the development occurring after separation could be either "obligatory or facultative" based on his observations that one subset of cells generally continued to develop, another subset of cells could be helped to develop, and a final subset died. He decided the factors for continued development were determined by characteristics of the protoplasm, and not influenced by the nucleus. He also believed the protoplasm had polarity and bilaterality from the time of oogenesis (Driesch, 1908).

Driesch's initial experiments prompted this fundamental question. "Is the prospective value of each part of any state of the morphogenetic line constant, i.e. is it unchangeable, can it be nothing but one; or is it variable, may it change according to different circumstances (Driesch, 1908)?" With continued experimentation, he found a partial answer to his question in three observed phenomenon. First, where the gastrula was cut determined whether or not a whole organism would form. Second, the "prospective potency" of the ectoderm is not the same as the blastoderm, but is more restricted until there is no longer any prospective potency. And finally, the "prospective value of each cell is a function of its' position" (Dreisch, 1908).

Driesch's last observation, that the fate of a cell is a function of the position of the cell in the embryo has emerged as one of the main components of current pattern formation theories. However, his theories have been lightly considered by current researchers because of obvious errors, as well as misinterpretations of his hypotheses. Driesch was mistaken when he dismissed the importance of the nucleus in development. However for species with mosaic eggs, he foresaw the relevance of specific divisions of the cytoplasm during development. Driesch found that the single factor, calcium was necessary for development to continue at the eight cell stage; these results were later confirmed in experiments using mouse embryos (Whitten, 1971). However, Driesch questioned the ability of a chemical factor to determine the course of development. He decided the unknown directing factor for development was present within the zygote. For this reason, he borrowed the term "Entelechy" meaning "bears the end in itself" from Aristotle. Unfortunately, he described the mechanism as the process of vitalism in keeping with the current thinking of his time (Driesch, 1908). Vitalism has been interpreted to denote a mysterious life force (Wolpert, 1991, Bryant, 1982), and this interpretation has contributed to the dismissal of Driesch's hypotheses, excepting minor credit for the theory of cell position as a factor for cell fate.

Hans Spemann also studied the effect of position on cells in regulative embryos in the early 1900's. Mangold,

from Spemann's laboratory, transplanted cells from the dorsal lip of a newt embryo into the blastocoel of an early gastrula and found a secondary axis frequently formed (Loomis, 1986). After Spemann, interest in pattern formation in embryological systems was sporadic. A resurgence of interest occurred in the 1960's. It was at this time that Wolpert presented his concept of positional information as a solution to the French Flag problem.

CONCEPTS OF PATTERN FORMATION

A. The French Flag Problem

In an effort to concretize a complex three-dimensional developing organism to a manageable construct, the French Flag problem was presented. The flag is composed of a field of approximately 50 cells (Wolpert, 1969) in parallel lines. The cells are to generate the pattern of a blue band, white band, and red band. One solution would enable the cells to learn their position in the field with respect to the boundaries of the field (Wolpert, 1969). This information is then interpreted and the decision is made to turn a specific color. The interpretations would depend upon the developmental history and genetic makeup of the cell. The cells would constantly monitor their position. If deletions or additions of cells were made early, adjustments of a cell's color or size of the flag could be made to preserve the pattern.

Wolpert suggests possible means of measuring the distance from the boundary. A chemical gradient with a fixed concentration at one boundary and a decreasing concentration as the morphogen diffuses across the field could provide the cells with information as to their position in the field. The cells would then respond to a specific concentration threshold by changing color. By varying the number of morphogens, and significant concentration thresholds, as well as the diffusion rates of the morphogens, increasingly complex patterns would be generated. Another possible mechanism for determining position would be for the cells to communicate with one

another by gap junctions or a chemical message. The extracellular matrix is also a potential source of positional information (Wolpert, 1991).

B. Positional Information

Positional information first described by Dreisch, was expanded upon by Wolpert in the 1960's. Wolpert defines positional information as the information specifying the cell's position in reference to one or more coordinates of a system. For a system to use positional information, a set of rules defining the order of the system is necessary, such as in a dictionary (Wolpert, 1984). Within a system, a field is a group of cells with the same set of reference points for specifying the positional information. The cells in a field must have the capacity to communicate with one another to effect regulation of the field (Wolpert, 1969). The cells also require polarity to determine the relevant direction from which to measure the information. The mechanisms for specifying positional information are universal across the developing embryo and different species (Wolpert, 1984).

These aspects of Wolpert's concept of positional information are not unique to Wolpert's theory of pattern formation. The concept that a cell knows its' position in relationship to the surrounding cells by receiving information from the environment is prominent in most current pattern formation theories. Distinctions between theories are expressed in variations of the determination and expression of polarity of the cells, and the mechanisms by which the

positional information is transmitted to and interpreted by the cells.

C. Polarity

Polarity is defined as the direction from which the positional information is measured (Wolpert, 1969). Polarity specifies the cells and components of cells to receive the information first. The polarity of cells in a field could be graded with a high to low potential correlating with the direction of the positional information. For either a unipolar or bipolar system only one polarity is predicted with the polarity measured in one or both directions (Wolpert, 1969). Wolpert suggests the polarity is set by either a increase or decrease in the chemical gradient which concurrently specifies positional information (Wolpert, 1984). For the past 20 years numerous experiments have been carried out to identify a morphogen in a system such as described by Wolpert. A possible candidate for the morphogen is retinoic acid (RA). Results of studies with RA have varied results. In the chick limb, application of RA can induce extra digits (Summerbell, 1983) or cause limb reductions (Kochhar, 1973). Recent studies propose that RA establishes a field for the primary body axis, but does not specify graded positional information (Bryant & Gardiner, 1992).

Cells can have polarity based on the transcellular ion currents following through their plasma membranes. Steady ion fluxes through the cell generate gradients in the nanovolt range. These currents flow through ion channels which are

unequally distributed around the plasma membrane (Jaffe & Nuccitelli, 1974). The ion currents are correlated with the axis of cell polarity. In the *Xenopus* zygote, transcellular currents are initiated at the time of fertilization and last for 3 minutes thereafter. The current enters at the site of fertilization and spreads over the egg in a wing shaped wave (Kline, Robinson and Nuccitelli, 1983). Calcium is suggested as the ion generating the gradient and subsequent polarity in animal cells (Jeffery, 1982). Ion currents are present in chick chorioallantoic membrane from 6 to 10 of incubation before evidence of structural or functional polarization. The potential of the membrane increases from -3 to -18mV during this time (Stewart & Terepka, 1969).

The possible role of the extracellular matrix generating tension to determine the polarity of a cell is also being studied. At approximately stage 15, the limb mesenchymal cells of mouse embryo shift their orientation toward the basement membrane of the epithelium by 28%. The shift is measured by the movement of the Golgi apparatus. Coincident with the shift of the polarity is an apparent increase in the amount of extracellular matrix (ECM), specifically in area beneath the basement membrane (Holmes & Trelstad, 1977). Polarity may also be set when the cells of the chick blastoderm secrete ECM which generates tension in the area opaca prior to changes cell form and position. When this tension is increased abnormal gastrulation occurs (Kucera & Monnet-Tschudi, 1987).

At the present time more research appears needed to determine the mechanism or mechanisms for setting polarity.

D. Models of the Mechanisms of Positional Information

Turing was the first to construct a mathematical model to explain pattern formation. By accounting for most of the known biological features in a current "state of the system", he devised an algorithm to project the mechanism by which the next state of the system could be predicted. His algorithm omitted the variables of internal cell structure and electrical properties of the cell; and concentrated on the chemical aspect of patterns formation. He stated that a stable homogeneous entity would always be subject to disturbances of varying natures. This instability precipitated the development of a pattern of morphogen concentrations (a reaction) in the form of a wave (diffusion). If the instability occurred in only one cell, and one or two morphogens created a stationary wave, he projected a dappled pattern formed. Three or more morphogens produced traveling waves. Turing stated his linear equations were unable to encompass the biological complexity of the developing system and predicted a more complete model would be developed on the digital computer (Turing, 1952).

Meinhardt developed the computer analysis for achieving pattern formations predicted by Turing. Meinhardt, like Turing, postulates an asymmetry in the relatively homogeneous developing system triggers the developmental process. The asymmetry is precipitated by a nonspecific

stimulus such as a change in temperature, or pH level. In an activator-inhibitor system, the cells produce an activator which promotes its own production (autocatalysis) and the production of an inhibitor. The activator is slow diffusing and short-range, the inhibitor is rapidly diffusing and long-range (Meinhardt, 1984). If the field is larger than the range of the activator and inhibitor, then the pattern will be a periodic one. In contrast, if the field is smaller than the range, the pattern will be a monotone with a high concentration at one end and a low concentration at the other (Meinhardt, 1982). In a simple activator-depletion model, depletion of the substrates necessary for producing either activator functions to limit the pattern formed (Meinhardt, 1982).

The activator and inhibitor in these systems are hypothesized to be morphogens. The inability to identify the morphogens and thereby know their chemical properties, forces this model to postulate conditions for pattern formation which have limited biological confirmation. The computer can generate a pattern which occurs frequently in nature. However, more than one program can be written to form the pattern depending upon the properties of the morphogens. At this time computer models can not definitively determine the mechanisms for pattern formation. Computer simulations can provide a means for testing complex models.

E. Interpretation of Positional Information

The positional information is generally accepted to

be used by the genes to determine a course of action. The information is interpreted according to the cell's genome and developmental history. The mechanism to affect the action of the genes is not yet defined. A change in the concentration of morphogens (Meinhardt, 1978) or ions (Graudine & Weintraub, 1982) are suggested gene activating factors, as are changes in receptors on the plasma membrane (Hood, Huang, & Dreyer, 1977).

DEVELOPMENT AND PATTERNS IN THE EMBRYONIC CHICKEN LIMB

The embryogenesis of avians and in particular, chick limbs, is amenable to direct observation and experimental manipulation and is the subject of numerous studies. The limb is composed of vascular, neural, muscular, connective and epithelia tissues which form intersecting patterns and allows for the study of pattern formation of several types of tissue in one subject. This section will describe the basic development of the chick embryo and patterns of the limb. Development of other vertebrates limbs is comparable to the development of the chick.

A. Epithelia, Mesenchymal, and Vascular Development

i. Stages 1 to 6

The period of gestation for the chicken is 20 to 21 days and is divided into a series of stages identified by Hamburger and Hamilton in 1951. The stages are designated on the basis of external characteristics such as heart formation, number of somites, and limb shape; and are the developmental standard for current experiments. During the first six stages the primitive streak, Henson's node and the head fold form (Hamburger & Hamilton, 1951).

ii. Stages 7 to 25

The limbs arise from the medial and lateral somatic mesoderm (Geduspan & Solursh, 1992). The anteroposterior axis of the wing is determined before stage 8; the dorsoventral axis set around stage 11 (Chaube, 1959). The mesoderm defines the anteroposterior and dorsoventral axes; however the

dorsoventral axis of distal elements can later be altered at stages 19 to 22 by rotating the AER by 90 degrees (MacCabe, 1974). The wing develops first with an "inconspicuous condensation of mesoderm" (Hamburger & Hamilton, 1951) at the wing level by stage 15, and a thickened ridge at stage 16. By stage 17, the wing and leg are both distinct swelling of equal size with the wing extending from somite 14 to 20. The stage 17 limb has a simple capillary network derived from the sprouting of the aorta (Evans, 1909). On the anterior and posterior margins of the plexus are the marginal veins. Around the peripheral edge of the limb bud is an avascular zone of approximately 100μ (Caplan & Koutroupas, 1973).

The stage 17 mesenchyme is undifferentiated with a slight condensation of cells in the proximal region (Fell, 1925). The epithelium consists of an outer, or epitrichial layer and an inner layer which is composed of cuboidal cells proximally and pseudo-stratified columnar cells distally which form the apical cap (Saunders, 1948). The apical cap, designated the apical ectodermal ridge (AER), is induced by the underlying mesoderm at approximately stage 17 (Saunders & Reuss, 1974). The AER in turn maintains the mesenchyme and is necessary for continued outward growth of the limb (Saunders, 1948). Similarly the development of the marginal veins, the pattern of the vasculature (Feinberg & Saunders, 1982), and maintenance of the avascular zone (Feinberg, Repo, Saunders, 1983) is controlled by the AER. The cells at the distal edge of the limb (200-400 microns) directly under the AER are in an area designated the progress zone (Summerbell

& Lewis, 1975). These cells remain in a proliferating and undifferentiated state from stage 17-18 until stage 28-29 when normal epithelium replaces the AER (Searls & Zwillling, 1964). Removal of the AER results in truncation of the limb as a function of the degree of development at the time of removal.

At stage 19, the proximal mesenchyme of the limb bud is a widely spaced population of morphologically homogenous cells, except for an area of increased cell density in the periphery at the level of the prospective humerus (Fell, 1925, Singley & Solursh, 1891). The nuclei are approximately 4-8 microns and form the bulk of the cells (Fell, 1925, Ede, 1976). Electron microscopy by Ede et al. shows the cells are in contact through long, cytoplasmic processes with adjacent and distant cells, as well as the basement membrane of the AER (Ede, 1974).

As the development of the limb mesoderm progresses several patterns of activity occur. Cell death occurs in patches on the anterior and posterior borders proceeding in a proximal to distal sequence. These areas are the necrotic zones. In the central prechondral area, cell death occurs in small zones termed opaque patches, which also follow a proximal to distal sequence (Saunders, & Gasseling, 1962). The area under the AER is not involved in the cell death pattern, however removal of the AER precipitates cell death for a depth of 150-200 microns into the mesoderm from the distal tip (Rowe, Cairns, & Fallon, 1982). On the posterior margin of the limb bud is an area of mesoderm called the zone

of polarizing activity (ZPA). The ZPA progresses distally as the limb develops, thereby remaining in contact with the AER. Grafting this area to the distal anterior margin of the limb can result in the duplication of digits (Saunders & Gasseling, 1968). The grafted ZPA does not become part of the extra digits, but influences adjacent cells to form the extra digits (Tickle, 1980).

From stages 18-19, there is an overall decrease in mitoses superimposed by a proximo-distal gradient of increasing cell density (Summerbell & Wolpert, 1972). The proximal to distal mitotic gradient is formed when the cartilage condensations and muscles formations develop first in the proximal portion. Summerbell and Wolpert (1972) suggested increased cell density limits mitoses in vivo and is a possible mechanism for control of growth and pattern formation.

The proximal central mesenchyme of stage 22 limb buds appears to form condensed, circular arrangements of crescent shaped cells, first described by Fell (1925) as "precartilage condensations". The cells then become rounded, starting in the center of the condensation, and extending outward. The pattern suggests a "center" around which the cells aggregate. Concomitant with the condensation phase, many biochemical events occur. The level of sulphate incorporation is uniform throughout the limb mesenchyme until stage 22 when the level increases in the areas of condensation (Searls, 1965). In vitro studies of stage 22-24 limb bud cells show that as the

level of chondroitin sulfate increases, hyaluronic acid (HA) production decreases (Toole, 1972). The ectoderm secretes large quantities of HA (Solursh, Fisher & Singley, 1979) and the peripheral ECM (the avascular zone) is composed largely of HA (Singley & Solursh, 1981). Fibronectin and type I collagen are evenly distributed throughout the mesenchyme at stages 22-23. Until stage 25, fibronectin and type I collagen levels increase in the condensations, followed by a decrease as differentiation occurs (Dressau, von der Mark, H., von der Mark, K., Fischer, 1980). The differentiation of the cartilage elements from limb mesoderm continues in a proximal to distal pattern until approximately stage 36 or day 10 (Saunders, 1948).

B. Muscle and Nerve Development

The somites begin their migration into the limb at stage 15 (Kenyon-Mobbs, 1985). These cells have long filopodia which are found to associate with blood vessels. The migration of the muscles is dependent on the presence of the AER (Gumpel-Pinot, Ede & Flint, 1984). By stage 20 the cells are distributed throughout the central core of the limb bud. These cells form premuscle masses and migrate to the dorsal and ventral regions of the limb. The formation and migration of the premuscle masses is complete by stage 23 (Schramm & Solursh, 1990). The dorsal and ventral premuscle masses undergo a series of subdivisions which follow a very precise order to form the individual muscles. The forearm muscles are defined by stage 32 (Kieny, Pautou, Chevallier, Mauger, 1986).

The sensory and motor axons which originate in the spinal cord also migrate into the limb. The sensory axons have their cell bodies in the dorsal root ganglia. The motor axons are in the ventral horn. The axons invade the limb at stage 22 (Bennett, Davey, Uebel, 1980) and progress to the distal tip with the growth of the limb (Roncali, 1970). A surplus of axons infiltrate the limb, substantial loss of axons occurs and the final pattern of innervation is set by stage 35. The limb controls the developmental pattern of the axons. However the mechanism by which the axons set up the appropriate pattern is not known. One theory is the track theory in which the axons seek out tracks set up by the limb tissue (Bray, 1977). In the chick limb bud tenascin is deposited around the growing nerves and is of glial origin. The tenascin is suggested as a facilitator of the invasion of the axons into the limb (Wehrle-Haller, Koch, Baumgartner, Spring, & Chiquet, 1991).

C. Gene Expression in the Developing Limb

There is considerable evidence for expression of the Hox 4.4-4.8 genes and the CHox-7 and CHox-8 genes in the developing limb. For the Hox-1 homeogenes, the results of the few studies done, suggest they are also expressed as nesting patterns across the limb during development. The Hox 4.4 is expressed across the entire limb mesoderm at stage 25. The area of expression for Hox 4.5 is smaller and is not represented along the entire anterior margin. This pattern of a increasingly smaller area confined to the distal-posterior

region continues for Hox 4.6 through 4.8. The Hox-4 homeogenes are also expressed as early as stage 17 in the same pattern as at stage 25 (Izpisua-Belmonte, Tickle, Dolle, Wolpert, & Duboule, 1991). Removal of the AER freezes the expression of the Hox-4 genes to the level of expression at the time of removal (Izpisua-Belmonte, Brown, Duboule, & Tickle, 1992)

The expression of CHox-7 and CHox-8 is most evident at stage 19 to 23. The CHox-7 transcripts were localized over the entire progress zone, while the CHox-8 transcripts were found only in the anterior part of the progress zone. At later stages, after stage 26, the pattern of expression is similiar for each gene. The transcripts are localized at the anterior periphery, proximal and distal: and the distal posterior periphery. At stage 31, the gene expression occurs in areas around the cartilage. The expression of CHox-7 is greater quantatively than CHox-8 (Nuhno, Noji, Koyama, Nishikawa, Myokai, Saito & Taniguchi, 1992). CHox-7 expression is significantly reduced following removal of the AER (Ros, Lyons, Kosher, Upholt, Coelho, & Fallon, 1992).

The embryonic limb is an immensely complex structure. Patterns of process are used by the limb to simplify the developmental process. For example, muscle and cartilage cells form small masses or condensations before differentiation. And as the limb grows, the patterm of muscle and cartilage formation is repeated for each skeletal element. Components of the ECM are produced and degraded in conjunction

the process of cartilage formation. The ECM is distributed in a pattern which coorelates with the vascular arrangement. Pattern formation evokes patterns of process.

THEORIES OF PATTERN FORMATION IN THE EMBRYONIC LIMB

A. The ZPA/Progress Zone Model

Wolpert's theory for pattern formation in the limb is based on the positional information concept, with an accompanying reaction-diffusion mechanism and genetic interpretation. He proposes that the proximodistal coordinates for positional value are established by a mechanism based on autonomous change with time in the progress zone at the tip of the limb bud. A positional signal from the ZPA determines the anterior-posterior axis. This signal is interpreted by only the cells in the progress zone (Wolpert, Lewis, Summerbell, 1974). The cells in the progress zone are exposed to a chemical signal with a regular cyclical fluctuation in its' concentration. As the cells leave the zone, the number of cycles and concentration level is frozen in them. This information is used to decide which bone they will become (Wolpert, 1984). Interpretation of the positional information from the progress zone could correspond to the "frozen" expression of the Hox-4 genes when the AER is removed.

A reaction-diffusion mechanism is proposed for the generation of the anterior-posterior axis. The number of peaks in a cycle the cell is exposed to while in the progress zone determines the number of bones formed, one peak equals one humerus. The asymmetry of the bones is generated by the signal from the polarizing zone. The proposed signal is a diffusible morphogen. The lowest concentration specifies the

second digit, as this digit is the farthest removed from the signal source (Wolpert,1984). The experiments described above, in which the ZPA is grafted to the anterior margin are presented as substantiation of this theory. The proposed morphogen of the ZPA has not yet been identified.

In 1959, Chaube transplanted rotated portions of the flank of stage 7 to 12 embryos. A previous experiment had designated these areas as future limbs. The results showed the anterior-posterior axis was set before stage 8 (Chaub, 1959). The ZPA and proposed signal are not present until after stage 18. In set of experiments by Iten and Murphy (1980), anterior donor limb bud tissue was grafted to the anterior margin of a host. Supernumerary digits were formed in a pattern similiar to the limb buds with posterior grafts. In any of the experiments where a portion of one limb is grafted to another, the percentage of limbs forming extra digits may be as low as 40% and is never 100%, with normal or simply deformed limbs formed in the remaining subjects.

Rubin and Saunders (1972) performed a series of experiments in which a late stage ectoderm was placed over an early stage mesoderm and vice verus. The results demonstrated that any functional AER induced the appropriate outgrowth regardless of the relative ages of the ectoderm or mesoderm. Therefore, the signal from the AER is constant and the information for the proper proximo-distal sequencing of the skeletal elements must be programmed in the mesoderm.

Until the signal morphogens are identified, the

inconsistent results from the various experiments can not be resolved.

B. The Polar Coordinate Model

The polar coordinate model was originally developed to describe the process in the regeneration of limbs. This model is also based on positional information in terms of a two dimensional grid. The antero-posterior and dorso-ventral axis are set by the value (0 to 12, 0=12) given to a cell on the circumference of an ellipse. The proximo-distal value (a to e) represents the distance from the center of the grid. When cells with normally nonadjacent positional values interact, a process of intercalulation occurs. If a 9 interacts with a 5, then the cells produce progeny with the intermediate values. When a 10 is produced at the time another 10 is already on the circumference, then the cell moves to the next distal ring. This process is referred to as distalization rule. The shape of the limb bud brings nonadjacent cells closer, resulting in the intercalation (Javois, 1984).

Grafting experiments in which cells with normally nonadjacent cells are abutted have produced the rules of intercalulation. If a 11 is placed next to a 2, then cells with the values of 12/0 and 1 will be produced. The shortest route between the two values is always taken. If part of a limb is removed, the limb can replace the missing values if at least half the values remain. In wound healing, the ability to regenerate the missing parts depends upon the shape of the wound surface. With a cross shaped wound where five

values are present, intercalation occurs without distalization. (Bryant, French, & Bryant, 1981).

This model does not explain how a cell with one value discriminates between other cells with different values. Nor how each cell knows their own value. The model lacks a method to measure the values of the cells during experimentation. The process by which intercalation proceeds is not known. The authors of the model suggest further experiments for these answers.

C. The Information and Memory Model

In the process of researching the material for this paper, I have been impressed by Wolpert's quotes at the start of his chapters. The quotes refer to the passage of people through their lives. Each quote encapsulates Wolpert's concept of pattern formation and gives cells human qualities. Cells as humans is a thought worthy concept.

I wish to redefine two phrases before I describe my theory. Cell memory is the process by which a cell stores information received from the environment. The memory is cumulative and accessible. The memory can be drawn upon to interpret the current environment and determine courses of action. The memory of the cell is not unlike our own memory. The memories are most likely stored on the chromosomes. Activation of a gene may constitute a memory. I propose that chromosome has a mechanism for remembering which genes have been activated in the current cell and its' predecessors. The message of the memory is expressed by the cytoskeleton.

Cell lineage is a family tree for the accumulated information the cell stores in its' memory.

Other concepts of this theory are positional information, instability, and a reaction-diffusion mechanism. The mechanism which creates the instability to start the reaction-diffusion is tension. The tension exists between the cell and its environment. An increase or decrease in tension can trigger the reaction-diffusion process. The tension can be in the form of mechanical stress exerted by the ECM or oxygen or osmotic pressures. Another source of tension could be a change in the transcellular currents. The basic tenet being the cell knows a change in tension signals incoming information about its' surrounding environment or positional information. The information is translated and carried by the cytoskeleton to the nucleus. The cell's response is based on its' accumulated knowledge. Cells without sufficient knowledge could not remember what they had been and are more easily regulated. Differentiation would occur after certain pieces of information had been collected.

Muscle tissue from 10 chick were cultured with same age cartilaginous elements of the leg on collagen. After 6 days of culture, the muscles had elongated and moved into position around the cartilage elements. The final arrangement was remarkably similar to the in vivo arrangement (Stopak & Harris, 1982). One possible explanation for this result is that the cells had enough accumulated knowledge to interpret their environment and respond as if in vivo.

CONCLUSION

Wolpert is given the credit for reviving the study of pattern formation. He clearly stated the problems and proposed several new concepts. The trend in pattern formation research at the present is to conduct experiments around his concepts. Twenty years have been spent searching for the morphogens. The result is the creation of a possible bias in the experimental research. Alternative concepts need to be discussed and tested.

Wolpert's emphasis on pattern formation is warranted. Pattern as a form or process is an vital part of all lifeforms. Without patterns, life does not exist. In conclusion, the answers to the mechanisms of pattern formation appear to be distant, but a necessary goal.

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