

Chapter 1

INTRODUCTION

Biological Patterning: From Awareness to Understanding

The attitude of humans towards patterns of plants and animals has gone through several transitions during the cultural evolution of man. First, there was primarily a bewilderment of nature, and this bewilderment concerned primarily the physical world and less the biological nature. Later came an epoch during which the developmental stages that result in mature animals and plants were followed and described. Only subsequently were experimental procedures employed in order to understand the processes and mechanisms that control development and lead to the formation of patterns in tissues, organs and whole organisms.

The following sentences from an ancient poem exemplify the phases of awareness and bewilderment of nature. The poem, in total, reports the fascination with the physical world and the greatness and omnipotence of God. Less emphasis is put on animals and only minor attention is directed towards plants:

“... He causeth the grass to grow for the cattle and the herb for the service of man ... The trees of the Lord are full of sap; the cedars of Lebanon, which he hath planted; where the birds make their nests; as for the stork, the fig trees are her house. The high hills are a refuge for the wild goats; and the rocks for the conies ... O Lord, how manifold are thy works! ... So this great and wide sea, wherein are things creeping innumerable, both small and great beasts.”

(The Book of Psalms, Chapter 104, Hebrew Bible, King James version)

This Psalms chapter is attributed to King David, and was composed in about 1000 BC. Only several hundred years later did the

early Greek philosophers (e.g. Thales of Miletus, ca. 636–546 BC, and his Ionian school) divert from pure mythology to questions regarding the evolution of nature. About 200 years later another Greek philosopher, Hippocrates of Cos (ca. 460–370 BC), known mainly as a physician, handled the development of organisms by rationalization. He based his claims about the formation of organisms on the then accepted three entities of nature: heat, fluidity and solidity. About 60 years later emerged a “giant”: Aristotle (384–322 BC). Aristotle had a holistic approach that took into consideration the physical as well as the biological world. According to him there are levels of ascending entities: non-moving entities (e.g. rocks), plants, non-speaking animals and a speaking animal (man). Aristotle and his Lyceum school introduced the concept of causality. For him, man is at the “top” of the hierarchy. The other entities of nature exist to serve man, who is the ultimate goal of the universe; hence, Aristotle was what we call now a teleologist, attributing a strive or even an intention to entities trying to attain a goal. Moreover, the teleological explanation was for him the key for the proper study of organisms. If we take an example from the plant world, the development of an orange tree is meant to attain an orange fruit.

Attributing purpose to created entities did not start with Aristotle. It can be deduced already from the Psalms poem: “... grass to grow for the cattle ... cedars of Lebanon ... where birds make their nests ... high hills are refuge for the wild goats ... the rocks for the conies.” For the development of animals, Aristotle considered two possibilities. One was that mature animals are fully represented, structurally, in the young embryo. Then there is only growth and expansion. The other possibility, favored by Aristotle, was that new structures emerge during differentiation from embryo to adult. Aristotle did not deal only with principles but also dealt in detail with organisms, including the description of numerous plants. But, he was limited by the lack of “tools”, thus his descriptions were based merely on what the naked eye can see.

After Aristotle, the study of the development of organisms, including plants, went into “hibernation” for about 1900 years. Novel tools are the key to progress in biological investigations. This shall be exemplified in several chapters of this book. Hence, a new era in biological

investigation started in the early 17th century. In 1603 Federico Cesi founded in Italy the Lincean academy, the first post-Renaissance academy that intended to promote scientific knowledge. The name of this Academy was derived from the lynx, which was considered to have very sharp vision. The new tool that provided the Academy with a much sharper sight was the magnification lens. Lenses could be used to look at celestial bodies (by the telescope) and this was performed by a prominent member of the Lincean Academy, Galileo Galilei. Other members of the Academy utilized lenses (i.e. microscopes) to look into the details of organisms, including developing plants and their reproductive organs. The endeavors of the members of the Lincean Academy in the early 17th century heralded the beginning of the modern era of plant development.

Microscopic studies were not only performed by the Linceans in Italy. Microscopic observations were also conducted by others, such as Robert Hooke (1635–1703) of England and Antony van Leeuwenhoek (1632–1723) of the Netherlands. Here I would like to retrieve a wrong assumption that I made in a previous book (Galun, 2003). My assumption concerned the philosopher Baruch (Benedict) Spinoza (1632–1677) who was also born in the Netherlands but made his living from the polishing of magnification lenses. Born in the very same year as Spinoza, van Leeuwenhoek lived twice as long as Spinoza. My (humoristic) assumption was that the life of Spinoza was shortened from the dust of polishing lenses for van Leeuwenhoek's microscope lenses. I was wrong! For van Leeuwenhoek, who was a businessman, microscopic observations were a hobby. But, he polished his lenses by himself!

I shall not describe the progress in this field of science during the recent 400 years, and refer the interested readers to relevant text books, should they want to learn more about this. But, it should be noted that during the last 100 years additional tools have become available. I shall mention only some of them. About 100 years ago genetics became a useful tool. This was followed by biochemical genetics and the discovery of the structure of DNA and its role in storage, memory and replication of information. Subsequently a plethora of molecular biology tools were developed.

Moreover, microscopic and ultramicroscopic techniques were vastly improved and served as very useful tools in the investigation of development. Finally, computers were recruited for these investigations.

Using these tools in various combination contributed towards an increase of our understanding of development in general and to a fast progress in plant patterning studies. Notably, there was always an interaction between studies on plants and studies on animals. Tools and methodologies developed for plants (e.g. genetics, awareness of transposable elements) were quickly utilized in studies of animals and *vice versa*. Moreover, in some specific cases, investigators of animals (e.g. E. Coen, E. Meyerowitz) shifted from studying animal development to studying plant development and took their tools with them. As we shall see below, the development of patterns in plants differs from that in animals, but there are also common aspects and even homologous genes. Consequently, studies on pattern formation in plants can benefit from knowledge obtained from animal studies.

Recommended Previous Books and the Outline of this Book

There are several outstanding texts that deal with development and pattern formation in animals and plants. Of these, I would like to mention three. The first is a book that focuses on plants, by T. Sachs (1991). Much of this book is based on the author's own study of tissue and organ formation in plants. Albeit that this book was written over 15 years ago before very important tools, especially in molecular biology, were developed, it is still useful. As noted above, the tools are of prime importance to biological investigation, and in the past 15 years there has been a "jump" in our understanding of pattern formation. For example, Sachs' book was written before the rather elaborate process of floral member patterning became an intensive subject of investigation. Nevertheless, this book provides a good background to plant patterning and illuminates important aspects of this issue.

There are two other, rather different books, that deal with development and pattern formation. While being very different, the two books were published almost simultaneously and by the same publisher

(one by Oxford University Press and the other by both Oxford University Press and Current Biology). The book of E. Coen (1999), entitled *The Art of the Genes*, is rather unique: it recruits art, science and philosophy to explain pattern formation in plants and animals. The book provides information from genetic and molecular-genetic studies as well as uses the artwork of several masters (e.g. da Vinci, Dürer, Hals, Rembrandt, Velazquez, van Gogh, Picasso, Escher, Magritte and Robinson) to explain how a final, rather complicated pattern can evolve. Unfortunately, the figures, which are an important component of Coen's book, are without color, while color, as a metaphor, is a central issue for the deliberations of this book. The book is well written and has the typical flair of its gifted author. It stops short of providing detailed information on the development and pattern formation of the cells, tissue and organs of plants, but constitutes a very good introduction to these patternings.

The third book (L. Wolpert, 1998) has a unique authorship. The whole text was written by Wolpert, a world-renowned expert on development, but he was assisted by first-rate experts; moreover, each of the 15 chapters was reviewed by several specialists before being finally written by Wolpert. The many clear and colored illustrations in this book render even complicated issues understandable to the novice. The great majority of the book deals with animal development but there is also one chapter (out of 15) that is devoted to plant development. This chapter is based on publications until 1996 (with one exception, published in 1997). Since numerous and very relevant studies were conducted in plant patterning during recent years, an update and expansion on what is known in plant patterning is now desirable.

This book will start with an overview on patterning and indicate which are the main solved and unsolved questions in plant patterning. It will also point towards common and uncommon developmental regulatory mechanisms in animals and plants. The book is then divided into two main parts. Part A shall deal with basic issues: factors and regulatory mechanisms that are common to several types of cell-, tissue- and organ patternings. Plant hormones, plant cell division and cell cycles, and the plant's cytoskeleton will be briefly discussed. There shall also be a summary of plant phylogeny that led to flowering

plants (angiosperms), as well as a brief discussion on the emergence of “novel” genes that affect patterning (i.e. gene duplication followed by mutation and selection). Finally, the novel approach of *systems biology* and its relevance to plant patterning will be discussed briefly.

Part B deals with specific kinds of patterning, and the patterning of tissues and organs are described (e.g. embryos, roots, shoots, leaves). The regulated transition from vegetative shoots to reproductive shoots (inflorescences) that is unique to angiosperms will be elaborated. Finally, the patterning of the typical organ for sexual reproduction in angiosperms, the flower, is handled in detail.

An Overview of Biological Patterning

By “biological patterning” it is meant the patterning of specific individual cells, groups of cells (e.g. tissues), organs, and whole organisms. In this book these patternings will be related to cells, tissues and organs of flowering plants (i.e. angiosperms). The awareness that all organisms are composed of cells emerged from the studies and rationalizations of the German botanist, Mathias Jakob Schleiden (1804–1881), and the German physiologist, Theodor Schwann (1810–1882). Although stationed in different universities, they arrived (in 1838/39) at the same conclusion: that all plants and animals are composed of cells. It was subsequently stated that all cells are derived from the division of previous cells. The conclusion of Schleiden and Schwann and the subsequent statement became known as the “Cell Theory”. This theory was then the cornerstone of studies on the development of organisms.

In angiosperms, where cell movement is extremely rare (it occurs during pollination) and can be neglected, patterns are the results of one or more of the following cell activities:

Cell division: Cells may stop dividing or divide at various frequencies and with various durations of the cell cycle; the division can either be into two similar cells, or asymmetric, resulting in cells of different sizes and forms; the plane of the division may be at different angles.

Changes of cell form: After division, cells may either become approximately isodiametric or attain specific forms, such as very elongated (e.g. the pollen tubes and cotton-seed hairs that can reach a length of several centimeters); their periphery may be smooth or highly indented (as in leaf-epidermal cells that appear as pieces of a jigsaw puzzle).

Changes in the composition of cells: The cells' contribution to pattern is dependent on the cytoskeleton (this will be elaborated in Part A of this book); the cell walls may attain different compositions and shapes, as is typical of tracheal cells.

Termination: Cells may attain their fully differentiated state as leaf-mesophyll cells that carry out the photosynthesis. Under regular conditions these are “terminal” cells that will cease further division, although after their isolation (as protoplasts) and culture, they can resume cell division (see Galun, 1981). Similarly, leaf cells, covering the mesophyll layers, and which under natural conditions are terminal, may be induced to rejuvenate in tissue culture, divide, and form flower-like structures. Terminal cells can be “eliminated” as root-cup cells that are shed to the soil.

Plant cells have three characteristics by which they differ from animal cells. In animals there is a clear separation of cell lines at a very early stage between cells that will form the sexual organs and produce the gametes and the rest of the cells that will form the vegetative organs of the animal. There is no such early “division of labor” in angiosperms. Another feature that separates angiosperms from animals is the omnipotence (or at least pluripotency) of plant cells. This is exemplified by the stem apical meristems that can yield, after due cell division, either vegetative or reproductive organs. The third characteristic feature of plants is that their cells are (almost always) surrounded by a rigid cell wall. Such cell walls do not exist in animal cells.

The Wolpert book, which deals primarily with animal development, introduced the various model systems by providing a “conceptual tool kit”. The book focuses on animal embryos to indicate that development is the emergence of organized structures from an initial,

very simple group of cells. The development is thus divided into five processes. These developmental processes are neither independent of each other, nor strictly sequential. The processes are classified in the animal embryo but human embryos served also as a reference. The first process that follows fertilization is a period of rapid cell division without increase in the total mass of the embryo. The second process of development consists of a spatial and temporal pattern of cellular activity in the embryo. The second developmental process also involves the laying down of the overall body-plan and the main axes of the embryo (e.g. anterior and posterior ends and dorsal and vertical sides). The axes can be seen as a coordination system to define locations in the embryo. This patterning process includes also the allocation of cells to the different germ layers (ectoderm, mesoderm and endoderm). The third process of embryo development consists of further morphogenesis and also involves cell migration. In the fourth developmental process differentiation of cells occurs, in which the cells become structurally and functionally different from each other. At least 250 clearly distinct types of cells were categorized in the human body. The fifth process is growth; meaning increase in size of tissue and organ initials that is produced by cell multiplication, increase in cell size and/or depositions of extracellular material. The above summary of embryogenetic phases can be recognized in birds and mammals; thereafter emerges the chicken or the newborn. This emergence signals, for animal biologists, the end of their main interest in development and pattern formation. Not so in angiosperms. It is the fully formed embryo (inside the mature seed) that is the starting point of the main interest of plant biologists in the patterning of angiosperms.

There is an additional feature that is of major importance in animal (embryo) development. This is the folding of sheets of cells. These foldings change the contacts between neighboring cells as in the formation of furrows during the differentiation of the neural system. Although recent studies in plants hint to communication between neighboring cells, the communications by which cells influence each other play a much more important role in animal differentiation. Similarly, programmed cell death (apoptosis) was revealed in plants,

but in animals apoptosis has a major role in the differentiation of organs (for example in the development of hands and feet it leads to the formation of fingers and toes, respectively). This brings us back to the importance of the Cell Theory. We can now pose developmental questions in a more focused manner: How are genes controlling cell behavior? Is it possible to relate cellular behavior to gene expression? While this question may be regarded as simple, the answers are very intricate. We shall learn, during further discussions, that cells of the very same animal or plant react differently to the same gene products! Cells in different tissues and organs are already programmed in different manners, and this different programming renders them to react differently to the same additional gene product.

Wolpert (1998) stressed the distinction between genes that encode proteins that have “housekeeping” activities (as enzymes involved in the cells’ metabolism) and genes that encode proteins that are tissue-specific or cell-specific and thus make cells different from one another. The latter proteins are the major players in differentiation and patterning while “housekeeping” proteins have no such roles. In both cases the levels of the respective proteins in a given cell is determined by what is generally termed “gene expression”. But, behind this term are a plethora of mechanisms that control this expression. Gene expression shall be elaborated in Part A, below. How many genes are involved in the development of animal embryos? Wolpert (1998) gave rough estimates. His estimate was that for fly and vertebrate embryo-development there are between 1000 and 50,000 such genes; and he mentioned that the total number of genes estimated to be encoded in the human genome is about 80,000. These estimates clearly show that important genetic information was added since Wolpert’s book was published, because after the human and the mouse genomes were sequenced, the total number of genes in the genomes was found to be less than half of Wolpert’s estimate. Hence, the complexity of an organism is not only manifested by the number of its genes. Some plants, for which the genomic sequence is already established, have not much less genes than humans. Surely the number of genes is not the only measure of the ...complexity. This is an emerging issue, and outside the scope of this book.

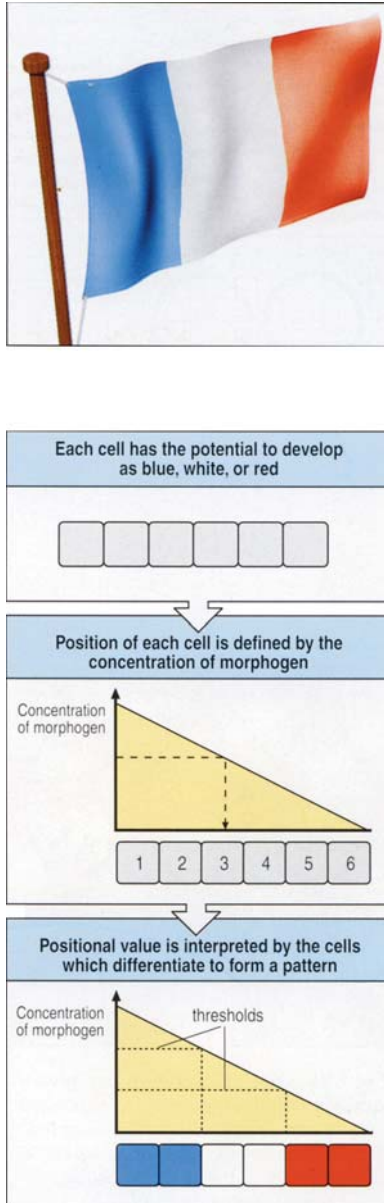


Fig. 1. The French flag model of pattern formation. Each cell in a line of cells has the potential to develop a blue, white, or red color. The line of cells is exposed to a concentration gradient of some substance and each cell acquires a positional value

The “conceptual tool kit” of Wolpert deals also with a model for pattern formation that is based on the French flag (see Fig. 1). It shows a color-based solution for position information that leads to acquisition of an *identity* and a *positional value* by cells. To demonstrate this model, a line of six cells is exposed to a gradient of a morphogen so that cell number one in this line is exposed to the highest level of the morphogen; the last cell in the line is exposed to the lowest level of the morphogen. The level of the morphogen is thus interpreted by the cell as its position. The two cells that are exposed to the highest morphogen level now “know” that they are at the anterior end of the line and differentiate into blue, according to the predetermined genetic program. The two cells exposed to the lowest level “know” that they are at the posterior end and differentiate into red.

Hence the model involves two requisites: the cells perceive their position by the morphogen level, and the cells have the capability to differentiate a color according to their position. This model also requires a threshold, meaning that as the level of morphogen is reduced gradually, there is an abrupt change in response (from blue to white and from white to red). This model seems abstract and not relevant to real patterning. But it is readily applicable for understanding patterning. We shall see, in the chapter of Part B that deals with the patterning of angiosperm flowers, that Coen and Meyerowitz (1991) applied a basically similar model to explain the differentiation of the four kinds of floral members in the model plant *Arabidopsis thaliana* (*Arabidopsis*). The patterning of *Arabidopsis*’ floral members will be discussed at some length in Part B, Chapter 12.

defined by the concentration at that point. Each cell then interprets the positional value it has acquired and differentiates into blue, white, or red, according to a predetermined genetic program, thus forming the French flag pattern. Substances that can direct the development of cells in this way are known as morphogens. The basic requirements of such a system are that the concentration of substance at either end of the gradient must remain different from each other but constant, thus fixing boundaries to the system. Each cell must also contain the necessary information to interpret the positional values. Interpretation of the positional value is based upon different threshold responses to different concentrations of morphogen. (From Wolpert, 1998.)

The book of Enrico Coen (1999) intends to lead the reader to a basic understanding of “how organisms make themselves”. We are fortunate that in Coen we have a combination of a talented and prolific scientist who has a keen interest and knowledge of art and philosophy, as well as the capability to write clearly for a wide range of readers. Before handling specific cases of development in animals and plants, Coen elaborates on his central metaphor. He leads the readers to the recognition that pattern formation in organisms is akin to an elaborate drawing executed by an artist on a canvas. The artist starts with a sketchy image and in a course of interactions between the existing drawing and the artist, the drawing passes through a process of additional details until the final, rather elaborate, picture is created. Hence, an organism is not the result of meticulously following a pre-existing recipe or blueprint, as happens in the manufacture of a car, but rather a *creative* process of interactions. Only, in the development of an organism there are not two separate entities: the artist and the picture; in development, the organism is capable of *creating itself*.

To clarify another aspect of development in animals and plants, Coen returns to the metaphor of car manufacture. A car can be produced on the basis of a detailed manual of instructions. But for that a person is required who can read, understand and interpret those instructions. Obviously, the instructions themselves cannot interpret the instructions; the latter shall therefore not be able to manufacture the car. But an egg can manufacture its mature image. As for cars, manufacturing instructions and interpretation of the instructions by production engineers, I had a lesson in 1961. I was a post-doctoral fellow at the California Institute of Technology in Pasadena. Together with other post-doctoral fellows, I was invited to visit the assembly line of Ford cars in Southern California. We saw the huge hangar in which different car components were moved, overhead, to the main assembly line. It looked like many streams flowing into a main river. It was clear that each stream carried the same type of component (e.g. doors, wings, bodies) but the components were not identical in shape and color. When we reached the exit of the assembly line, the sequence of cars coming out was surprising. The cars were of different models and of different colors. We expected that, for some hours,

exactly the same model and colour would exit the assembly line until another model would be assembled. The guide of the Ford company told us that the assembly line is marketing-oriented: each agent of Ford cars submits his order — an order may contain 20 different cars — and the assembly line is producing the cars order by order. We asked: “Why are there no mistakes, and does the ‘stream’ not occasionally bring a yellow door to a red car in the ‘river?’” The guide had a short answer: “We don’t have engineers who make such mistakes”; meaning that an engineer who makes a mistake in interpretation of the production instructions is fired right away. Well, coming back to biology, a mistake in the instruction or in their interpretation of patterning that concerns a vital tissue will kill the respective organism or prevent its ability to propagate. Nature, just as a manufacturing company, acts without mercy!

If an organism is likened to a computer, we have a case that the program (the software) is responsible for producing the hardware. In computers the software is completely separate from the hardware. And the hardware (the machine) has to be there *before* one can run the software, which has the instructions for building the hardware. We reach an impossible circular situation. In the development of an organism the program is recorded in the genome and the hardware is the organism itself. In the case of computers the solution is easy: the basic hardware is man-made. But organisms have to perform the development by themselves. One way out of this is to imagine a computer that can build itself, meaning that its software and hardware are interdependent. At least by some complicated mechanism such an imaginary computer would be able to modify itself. But this idea does not appeal to Coen, and he came up with an original idea of how development really proceeds; he contemplated how humans make things, and by “things” he primarily thinks of the production of art. Hence, the production of a work of art by a human artist became the favored metaphor for the process of the development of organisms. The continuous interaction between the artist (or the composer or the poet) with what is gradually being produced, does not serve Coen to *explain* development, but rather is intended to furnish a *viewpoint* that shall guide the reader through the intricate process of development,

in order to better come to grips with the elaborate scenario of plant patterning.

An important facet for understanding development and patterning is a knowledge of what is happening inside the cells that undergo this process, meaning primarily the ability to follow in detail the changes in the activity of genes (and changes in the levels of gene products) that are causally related to stages of pattern formation. The molecular-genetic tools that enable such a follow-up started to become available only in the past three decades. These tools are still being improved by computer-assisted methodologies. Some of these tools are mentioned below (Part A), but it is recommended that readers, who are novices in this field and wish to acquire detailed knowledge on molecular-genetic methodologies, study relevant texts.

We shall witness throughout this book that two different cells may respond in very different ways to the very same outside stimulant. This means that a cell may be predisposed in such a way that it reacts to a given affector in a unique manner, or even does not react at all. For example, a mesophyll cell will not enter into a new cycle of cell division when exposed to a morphogen (e.g. a plant hormone, such as an auxin or a cytokinin) while the same morphogen will induce division in some other cells. Likewise a specific cell in the shoot apex of a stem may respond to a given protein (e.g. a transcription factor) that is furnished internally, while another cell at a slightly different location will show a different response to the same protein, or may not respond at all. We shall meet such specific predispositions of cells in examples of plant patterning that shall be detailed in Part B of this book.

The term *morphogen* will appear during several discussions of patterning. The term has a functional meaning: these are substances that flow and can affect the behavior of cells, or even specific regions of a cell. But, chemically they may be either defined or undefined because not all of them were characterized. The characterized morphogens are of very different kinds. They may be plant hormones (as noted above), messenger RNAs (mRNAs as in fertilized fly eggs), or proteins and protein complexes. The term was actually coined by a mathematician/computer expert, Alan M. Turing. Turing was a first-rate

mathematician and a pioneer in computer science. He became famous because of his role in the Enigma project, during World War II, that deciphered the German code. Later he returned to mathematics to study *Hydra* development, and used this model for the development of algorithms of flow mechanics. Indeed a 35-page paper suggested that during *Hydra* development there is a flow of a morphogen. But neither the morphogen nor the channel of flow were characterized at that time (1952). Soon after this publication Turing committed suicide.

Some morphogens, such as the growth regulators, auxin and gibberellins, flow unidirectionally from a considerably distant source; others, such as brassinosteroids, are produced nearer their target cells. It is plausible that the same cell is affected by more than one morphogen, arriving from different directions. It is easy to conceive that a cell can sense the level of a morphogen, but does the cell sense the *direction* of the flow of a morphogen? Let us assume that the cell is capable of sensing a *gradient*. If this is correct, then in a cell that is exposed to a gradient of a morphogen, one end of the cell's envelope will sense a higher level of the morphogen than the envelope on the opposite side. An imaginary line connecting the two ends will then indicate the direction of the flow. Precise, specific and accurate analyses of morphogens, at the cellular and subcellular levels, which are not yet available, should furnish answers to the above-mentioned questions. We shall recall that morphogens such as auxin do not "pierce" the plant cell as an arrow: entering at one end and coming out at the other end. Rather, there are receptors at the cell's membrane that are affected by the auxin and transfer (by a rather elaborate process, as shall be discussed in Part A) the signal into the internal volume of the cell. In general, the details of the reactions of plant cells to a flow of morphogens are still enigmatic.

Solved and Unsolved Major Issues of Plant Patterning

The details of the major issues in plant patterning that have already been solved will be presented in Part B of this book. Here I shall provide only an overview of them. First, the structural issue of plant patterning — at the levels of the naked eye, the microscopic

observation and the electron-microscopic observation — was solved; at least for model plants such as Arabidopsis and snapdragon (*Antirrhinum majus*). The intracellular structures, such as the cytoskeleton, were successfully analyzed; in many cases with the aid of specific labelling and confocal microscopy.

Gene expression, at the level of mRNAs and proteins was readily evaluated in cells and tissues. Major progress was made in the understanding of plant patterning by using genetic methods. By *forward genetics*, a plethora of mutants having specific phenotypes, concerning patterning, was isolated. Such mutants are appropriate material for further molecular and structural studies. In *reverse genetics* the investigator focuses on a certain sequence of nucleotides and can ask what role this sequence plays in such cellular activities as cell differentiation and plant patterning. This is done by mutating the specific sequence. There are various methods to achieve this. One method that emerged in recent years is RNA silencing (Galun, 2005a). In this method the sequence of nucleotides in the DNA is left intact but a specific transcript is destroyed (or very much reduced), or the translation of the transcript is strongly reduced. Also, the chromatin that encompasses a given nucleotide sequence may be modified to reduce the transcription of the respective nucleotide sequence.

The advantage of the RNA silencing procedures is that the silencing of a given DNA sequence can be regulated spatially (i.e. in specific cells and tissues) and temporarily (i.e. at different developmental stages). Thus the establishment of refined tools to investigate plant patterning is demonstrated in the combination of molecular-genetic methods, computer-based analyses and instrumentation that now enable us to screen changes in the expression of a vast number of genes in cells and tissues that undergo differentiation or have defined mutations. Such throughput analyses will be presented in Part B of this book, when dealing with specific cases of investigating patterning.

A final “solved problem” to be indicated in this overview is the availability of the full sequence of the DNA in the nuclear genome of several plants that serve as models for the study of plant patterning. Such genomic sequences are now available for Arabidopsis and rice (*Oryza sativa*); partial sequences are available for additional plants and

the full sequences of additional plants are expected. Moreover, the respective sequence data is readily accessible to all investigators. The functionality of these genomes is progressively being understood and is very useful for pattern investigations. In parallel, genomic sequences and their functionality are available already in fungal organisms and several animals, such as worms (*Caenorhabditis elegans*), insects (*Aedes* sp., *Drosophila melanogaster*), fish (*Denio rerio*) and mammals (*Mus musculus*, *Homo sapiens*). The respective data are readily available by computer search and are of great help to investigators engaged in the study of plant patterning.

As for unsolved questions of plant patterning, we have a logical problem: it is difficult to specify all the mysteries of systems with which we are not fully familiar. In Part B several systems of patterning in cells and organs of plants shall be handled, and it will become evident that a lot of knowledge on these systems has been accumulated in recent decades, but in no system do we possess a complete understanding. A central enigma was already alluded to above when the basic ideas on development, put forward in the books of Wolpert (1998) and Coen (1999), were briefly reviewed.

It became evident that for a proper participation of a cell in patterning, the cell has to be *predispositioned*. Only predispositioned cells will respond correctly to internal and external effectors. Predisposition can take place only after the cells “know” their position in space (relative to other cells in their immediate and/or less immediate surrounding) and time (the developmental phase). How does a cell get to “know” its position? Wolpert (1996), in a “Perspective” review, addressed the issue of positional information in animals that was later elaborated on in his above-mentioned book (Wolpert, 1998). But clearly this question is still far from a complete answer.

I would like to mention one exception. The brown alga (*Fucus*) releases egg cells from the fronds of female plants and sperm cells from fronds of its male plants. Fertilization takes place in the seawater of the ocean (and can be performed in a Petridish). The fertilized egg cell settles on the substrate (rock, or the bottom of the dish). When polarized light is illuminating this egg, the predisposition takes place. Consequently, apical hairs will develop at the side of the egg that faces

the source of light and rhizomes will develop at the opposite end of the fertilized egg. Interestingly, the fertilized egg “remembers” its orientation during several cell division cycles. This is evident because the development of apical hairs and rhizomes can be inhibited by high osmotic pressure in the medium. The high osmotic pressure does not inhibit cell division. When later the osmotic pressure of the medium is reduced, the apical hairs and the rhizomes develop at the sites that are expected from the light exposure (Galun and Torrey, 1969; Torrey and Galun, 1970). This is apparently a simple system of sub-cellular orientation, because a simple physical stimulation is involved; but in reality it requires an elaborate mechanism of light perception by cell membranes and further transduction of the light signal. Light and proper osmotic pressure are probably required for causing the predisposition that initiates the molecular-genetic process that leads to the development of apical hairs and rhizomes. After the fertilized egg cell “knows” that it has a dark substrate below it and sunlight from above, it is able to develop the rhizomes and the apical hairs at the proper sites.

When we are concerned with a group of cells, as in the apical meristem of a plant shoot, we are faced with another yet unsolved question: how does a cell know that it is located at a certain place in this meristem? It can be at the very upper tip or at the flanks of the apical dome, and consequently will be predisposed differently. We do have clues, such as the flow of auxin, but not detailed knowledge. One reason for the lack of precise knowledge, with respect to auxin flow, is that present analytical methods do not furnish a quantitative evaluation of auxin levels in individual cells. Notably, such an evaluation is possible for many defined proteins (as transcription factors) or RNAs (as mRNAs).

We should remember that for understanding development, it is not only important to know when and how the expression of specific genes is activated but also when and how the expression is stopped and the gene products are eliminated.

The importance of the correct sensing by a cell — *where* its position is — is central in patterning. A few examples will help to demonstrate this importance. For a cell to start differentiating into a trichome, it has to sense that it is a leaf-epidermis cell on either the

upper (adaxial) or lower (abaxial) side of the leaf. Also, a cell has to sense that it is a leaf-epidermal cell before it will initiate division towards the formation of stomata. For other kinds of differentiations, such as the formation of tracheal cells, a cell must sense that it is located in a file of cells projected from the root or the shoot apical meristem that is destined to become vascular tissue. Likewise, in order to become predisposed for the participation in the formation of a floral member (as sepal, petal, stamen or carpel), the cell has to sense *where* in the dome of the reproductive shoot apex it is located.

How do cells achieve such a sensing of their location? In most cases this question does not yet have a full answer. We do know that cells communicate by sending signals to each other and in some cases (as shall be detailed in Part B), protein, RNA or hormonal signals were identified. In specific cases signals can move a long way, as in the defense against viral infection, when the movement of RNAs complexes were found to travel along the plant. In other cases, as photoinduction of the change from vegetative shoot apices to reproductive apices (i.e. inflorescence), there are indications of a movement of a signal from a photo-induced leaf to the shoot apex. But the signal is not fully characterized. Long- and short-distance movements of RNA and proteins in plants can now be traced by elegant procedures (see Kim, 2005 and Aoki *et al.*, 2005).

In summary, while vast progress has been made in recent years with respect to plant patterning, there is a long way to go to full understanding. The central question — how a three-dimensional plant pattern emerges from linear information, stored in the chromosomal DNA — still awaits clarification. An almost full answer to this question does exist in a subcellular entity: the T₄ bacteriophage. The host of this phage is the *Escherichia coli* bacterium. Like other T-even phages, it has an icosahedral head (with a diameter of about 700 Å). The head consists primarily of three proteins. The phage contains also a long tail, a connecting neck with a collar and long “whiskers” (tail fibers) and a complex base plate (see Fig. 2). The head contains dsDNA, in which all the genetic information of T₄ is stored. Figure 3 demonstrates the genetic map of T₄ (since the publication of this figure, additional details were provided).

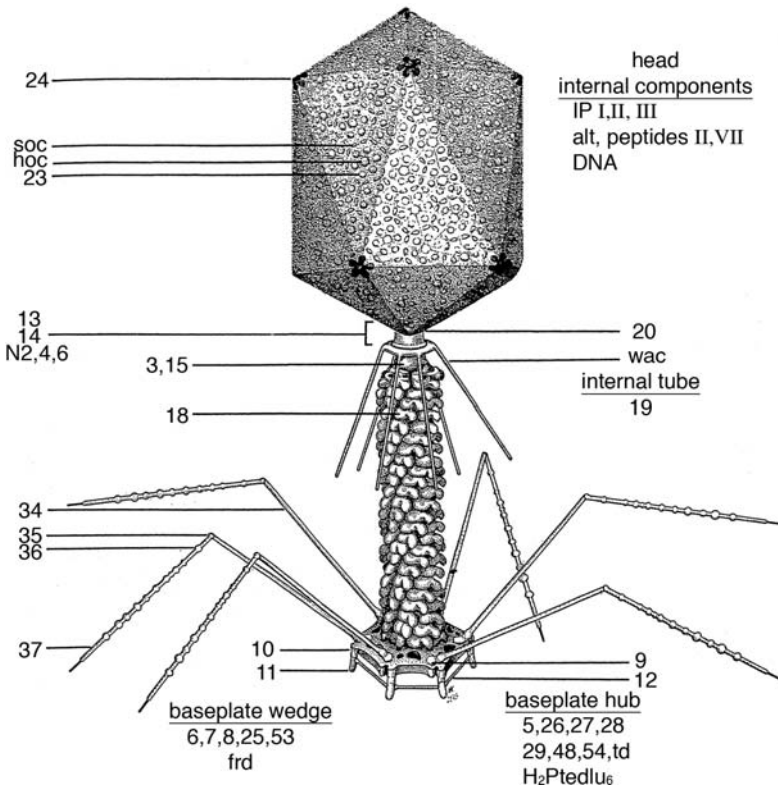


Fig. 2. Structure of the T4 virion based on electron microscopy at 2- to 3-nm resolution. The locations of protein components are indicated by gene number except for several unknown connector proteins, called N 2, 4, and 6, and the baseplate component dihydropteroyl hexaglutamate, called H₂Ptedl₆. The portal vertex composed of gp20 is attached to the upper ring of the neck structure, inside the head itself. The internal tail tube is inside the sheath and itself contains a structural component in its central channel. The baseplate contains short tail fibers made of gp12; these are shown in a stored or folded conformation. (From Mosig and Eiserling, 1988.)

During infection of the host bacterium, one or more of the tail fibers contact the lipoproteins of the host's cell wall and the phage is positioned perpendicularly to the surface of the host. The base plate approaches the cell wall, the tail contracts and causes the tube, which is inside the tail, to pierce the bacterial cell wall. The content of the head (mostly dsDNA) is then injected into the bacterial cell. Initially

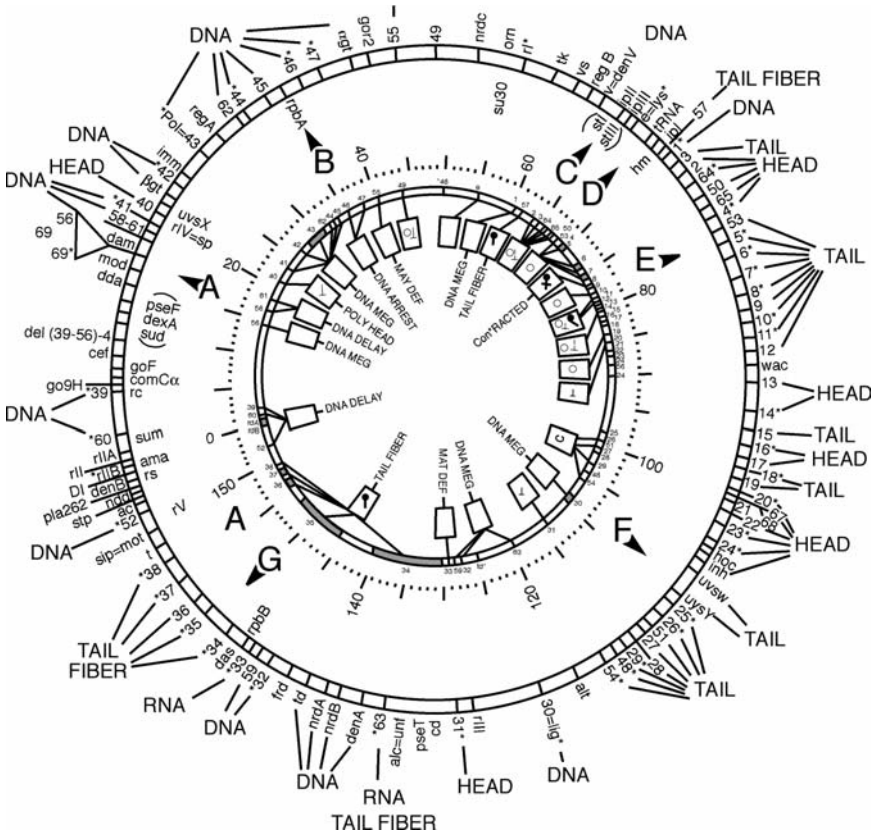


Fig. 3. A map of the known T4 genes. Distances are based on recombination frequencies (*inner circle*), on electronmicroscopy and restriction enzyme analysis (*middle circle*), and on the probability of packaging cuts (*outer circle*). The arrowheads labeled with large capital letters point to origins of DNA replication. (From Mosig and Eiserling, 1988.)

polymerases of the host are utilized by the phage, but with respect to all later metabolism and the construction of the phage progeny, the phage is autonomous and based on the genetic information of the phage.

The construction of new phage particles is a very elaborate process. In a way it is akin to the production of cars in the Ford assembly plant mentioned above. Different components, such as heads and baseplates with tails, are assembled separately and then put together until

the mature phages are constructed. Again, one can visualize the process as streams coming together into a main river. The process is exemplified by the assembly of the head (see Fig. 4) and the tail (see Fig. 5). The numerous proteins required for the construction of the phage components interact in an orderly manner and then the components are combined. All this is orchestrated by a set of several hundred phage genes, (almost) all of which were characterized and sequenced. But this orchestra is without a known conductor! Some additional details on the assembly, protein components and three-dimensional structure of T₄ are still being furnished by novel methods (such as cryoelectron microscopy). Despite this ongoing study, we already have a fair picture of how the linear information in the DNA

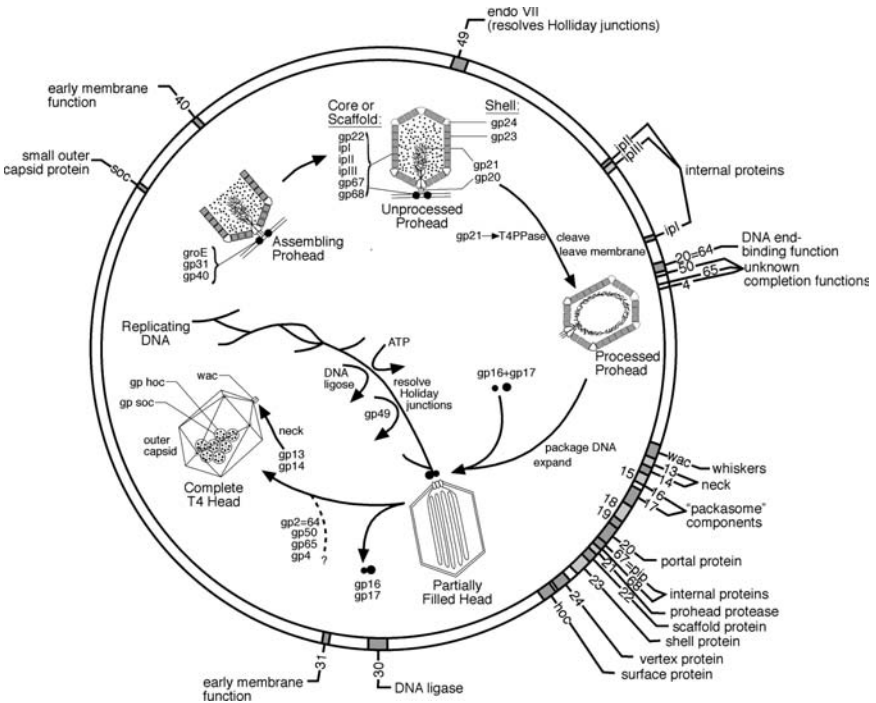


Fig. 4. Assembly and maturation of the T4 prohead and location of genes that control these processes. Interspersed tail genes are indicated on the inside of the circle. (From Mosig and Eiserling, 1988.)

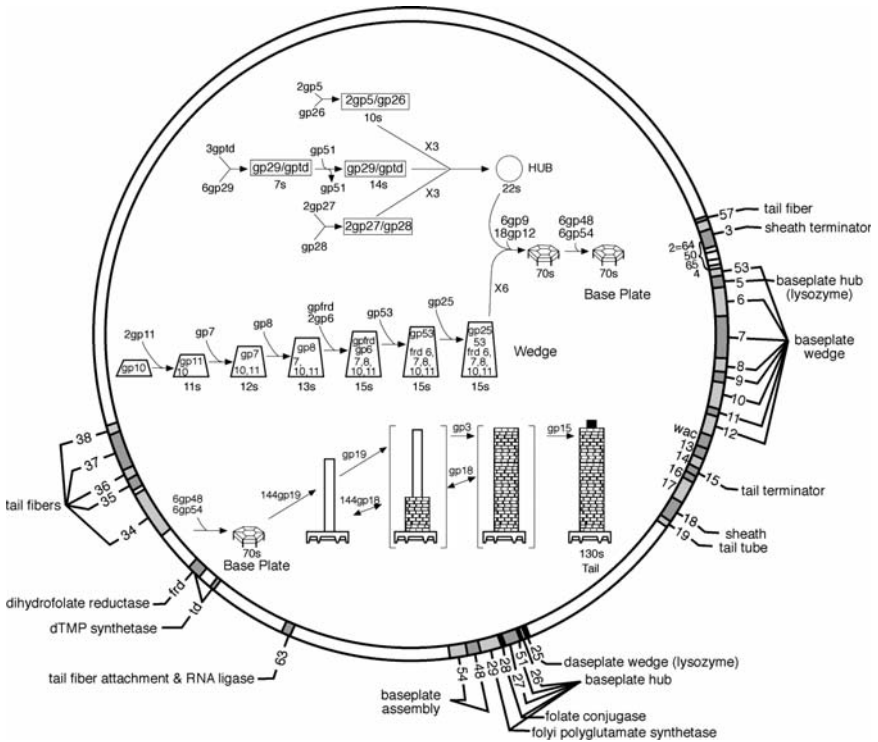


Fig. 5. Assembly pathways for both the hub and wedge parts of the baseplate, and the steps in the tail assembly, showing the positions of genes for tail and tail fiber synthesis and assembly. Head genes interspersed among tail genes are shown inside the circle. (From Mosig and Eiserling, 1988.)

of T_4 leads to a three-dimensional structure. Moreover, the thus constructed phage has several functional capabilities: it can recognize its host (*E. coli* bacteria), inject its DNA into this host, and is capable of replication.

Patterning in Angiosperms versus Patterning in Animals

Meyerowitz (2002) discussed the comparison between plant and animal development. He briefly traced the evolution of these two kingdoms of organisms as the basis of his consideration, and noted that the earliest signs of life on Earth are from about 3.8 billion years ago;

eukaryotic cells appeared about 2.7 billion year ago (as indicated by hydrocarbon biomarkers); the last common ancestor of animals and plants, which already went through the endosymbiotic inclusion of mitochondria from an alpha proteobacterium, is estimated to have evolved about 1.6 billion years ago. Thereafter was the phylogenetic split: the second endosymbiotic event was the uptake of the chloroplast from a cyanobacterium-like single-cell organism. Evidence for multicellular plants and animals indicates that these organisms emerged only after several hundred million years, about 600 million years ago. Therefore, the evolutions of multicellular plants and multicellular animals occurred separately.

By patterning, we mean development of multicellular structures. Hence, it is evident that the mechanisms that regulate patterning in plants and patterning in animals evolved separately. It is thus no wonder that plants and animals use different regulators for patterning. Nevertheless, animals and plants have some surprising similarities in the overall logic of development. A metaphor comes to mind. In my previous book I mentioned the metaphor of the mosque in Acre that was built by Achmad (Gàzar) Pecha, the Turkish ruler of Lebanon and Syria, in 1781. For building his mosque Achmad Pecha used different pillars that he collected from ruins of old Roman temples along the Eastern coast of the Mediterranean Sea. The old pillars were modified to fit their new purpose. This metaphor shall be coined as “The Pillars of the Mosque of Acre”. Here I shall modify the metaphor. Let us assume that a church was also built, north of Acre, and it was revealed that the builder of this church also used modified pillars from the very same ruined Roman temples. Now we have two rather different entities, but both are based on very similar components. Like other metaphors, this one does not fit exactly to reality.

As for patterning, an important component of plant patterning that we shall meet below are MADS box genes: a family of genes encoding transcription factors. These are master-regulatory genes that are active in several plant patternings as the specification of the radial pattern of flowers (in *Arabidopsis* and other plants). While very few MADS box genes were also detected in animals, the latter are not the common master-regulators of pattern in animals. Animals use family

members of the HOX homeobox genes for patterning. The latter genes have no sequence similarity to MADS box genes. Plants also have a few HOX-like genes, but again, these genes are not the master-regulators of plant patterning. It thus appears that both HOX-like genes and MADS-like genes existed in the last common ancestor of plants and animals but then they attained different roles in these two groups of organisms.

The metaphor on the mosque and the church and the ancient Roman pillars is thus relevant to the patterning of multicellular organisms. Meyerowitz (2002) listed several additional animal and plant proteins that have specific roles in the patterning of these organisms. The respective amino acid sequences in these proteins from the two groups of organisms can be similar, but the role of these proteins are different in these two groups. There are also proteins in plants that have a role in patterning but have no equivalents (in sequences or in roles) in animals. For example, there are no genes for phytochrome in animals. It is assumed that phytochrome-encoding genes were introduced into the plant genome by “horizontal transfer” from cyanobacteria about one billion or more years ago. These genes were then utilized in plants for the perception of red and far-red light, in a cascade of mechanisms that control growth and differentiation in response to red/far-red light.

Plants also contain genes that encode ethylene receptor proteins and no equivalents to these genes were found in animals. In plants, ethylene is a growth/differentiation hormone. Ethylene probably also has a role in pathogen response in plants. It was suggested that the ethylene receptor-encoding genes, also derived from cyanobacteria and by “horizontal transfer”, were introduced into the plant genome. As with the phytochrome genes, it is plausible that such a transfer could have happened after plants diverted from animals, but before they became multicellular organisms, about one billion or more years ago.