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The Molecular Basis of Sex and Differentiation

A Comparative Study of Evolution, Mechanism, and Control in Microorganisms

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Preface

Man's mind stretched to a new idea
never goes back to its original dimensions
Oliver Wendell Holmes

Our current understanding of sex and biological differentiation results from the application of three principal experimental approaches to these subjects: those of the physiologist, the biochemist, and the geneticist. These three approaches are illustrated by the materials presented in the chapters of this volume. Chapters 1–5 emphasize conceptualization of developmental processes, describing systems principally from the standpoint of the physiologist. Structures and functions are defined with only occasional reference to specific molecular details. Chapters 6–10 present the views of the biochemist, attempting to describe functions influencing or regulating cellular behavior at the molecular level. And Chapters 11–14 illustrate the approaches of the modern-day geneticist in his attempts to gain a detailed understanding of processes controlling gene expression.

While it is possible to delineate these three major sections, each emphasizing a distinct experimental approach, it must be realized that the yield of knowledge increases exponentially with the number of experimental approaches available to the investigator. Information resulting from the application of each of these approaches must converge to give the same answers for any one biological phenomenon in any one experimental system. Further, if we can learn of details regarding a particular process by applying different experimental approaches, our postulates concerning the underlying molecular mechanisms are likely to be more accurate.

But biological systems are not unrelated. Evolution provides the link that allows us to relate processes occurring in one organism to those operative in all others. A function is likely to be similarly performed in organisms that diverged relatively late in evolutionary history, while those diverging earlier will exhibit greater differences. With evolutionarily divergent organisms, mechanistic differences may have evolved to accommodate the differing degrees of complexity of cellular construction or to coordinate functions of differentiated cells in a multicellular organism. Still, we must realize that the basic life-endowing molecular processes had to exist prior to extensive evolutionary divergence—even

before the appearance of two distinct cell types. Consequently, we should expect that these processes are governed by the same principles, and that even the molecular details will sometimes be conserved throughout evolutionary history. Based on these postulates, it can be assumed that information available through the study of microorganisms will be directly applicable to higher organisms, and that molecular processes controlling complex interactions in multicellular organisms will have their rudiments in the essential life-endowing characteristics of the simplest bacteria.

This "unity principle in biology" is possibly the most important concept facing the modern-day biologist. It allows extrapolation of information from one organism to another as well as integration of basic facts obtained through the study of multiple species. Nevertheless, it does not preclude diversity. A given function may be performed by several distinct mechanisms, and a particular organism may have selected one such mechanism while another has chosen a second. Moreover, an organism may perform a function in more than a single way, and the two "pathways" may compete with, complement, or be superimposed on each other. Multiple pathways allow for fine control and permit more rapid evolutionary change.

This unifying maxim is tacitly applied by most developmental biologists. It is widely accepted that morphogenetic forces operative during embryogenesis (Chapter 1) will exhibit features common to those of developing bacteria and eukaryotic microorganisms (Chapters 2 and 5). The concepts of cellular and organismal mortality, to be contrasted with the potential for immortality in higher organisms (Chapter 3), have been formulated through studies with microorganisms (Chapters 2, 4, and 13). The similarities noted in Chapter 4 among cyclic programs of differentiation in prokaryotic and eukaryotic microorganisms and the molecular mechanisms underlying these programs (Chapter 5) also attest to a universal evolutionary origin.

As discussed in Chapter 6, microorganisms control their internal environment by restricting or facilitating the stereospecific movement of molecules across their membranes. Multiple mechanisms of translocation have evolved and most of these occur universally throughout the living world. Similarly, micromolecular and macromolecular reception processes as well as intercellular recognition (Chapters 7–10) are likely to be mediated by functionally analogous cell surface proteins and glycoproteins. These reception processes must trigger intracellular transmission mechanisms (Chapters 7 and 9), which elicit appropriate biological responses. Finally, the genetic material of the cell must be differentially articulated, depending on the stage of differentiation in which the cell finds itself. Owing in large part to the advent of rapid gene cloning and DNA sequencing techniques, the molecular details of genetic regulatory processes are now coming to light (Chapters 11–13). Again, our appreciation of the diversity of genetic regulatory mechanisms operative in the biological world relies upon the multiorganismal approach to the problem.

The present monograph is designed to familiarize the reader with the essential unifying concepts in developmental biology. In attempting to illustrate these

principles we shall wander to the edges of (and beyond) the frontiers of our knowledge. When possible, these concepts will be pursued to the molecular level. Extensive reference to experimental detail has been omitted in order to maximize conceptual recognition of the underlying principles governing cellular and organismal development. Only when a knowledge of experimental protocol is essential to understanding the process under discussion will this information be presented. Selected references at the end of each chapter are provided to allow the reader to pursue a subject in greater depth.

What is true for *Escherichia coli*
is true for elephants, only more so.

Jacques Monod

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Contents

CHAPTER 1

Embryology and the Study of Microbial Development

Thou art thy mother's glass, and she in thee
Calls back the lovely April of her prime;
Make thee another self, for love of me,
That beauty still may live in thine or thee!

Shakespeare

The study of embryology, and consequently of biological development, was initiated by Aristotle in 340 B.C. He followed and recorded the development of a chick embryo within the egg, noting that the developing embryo went through distinct chronological stages that resembled those of other organisms. From this observation stemmed the postulate that ontogeny is a recapitulation of phylogeny (Baer's Law).

Two principal antagonistic theories were put forth to account for embryological development: Preformation and Epigenesis. The Preformation theory stated that a preformed creature resembling the adult organism was present in the embryo throughout development; the epigenesis theory suggested the emergence of morphological features during development. With the discovery of sperm in the 1700s, the preformationists assumed that since the father contributed the "Creative Principle," the "little man" had to be contained within the sperm cell. The discovery of parthenogenesis in 1750 and the dissection of early embryos in 1760, revealing only fluid and granuoles (no little man), were severe blows to the preformation hypothesis.

In 1888, Roux, the father of experimental embryology, allowed a fertilized frog egg to divide, killed one of the two daughter cells with a hot needle, and observed the consequences. The morphologically defective embryo that developed resembled half of a tadpole. Preformation appeared to be supported. Roux did not attempt to remove the dead cell from the live one, and it was not until 1930 that the experiment was repeated with cells separated from 2-cell stage embryos. Each cell gave rise to a normal tadpole, thus dealing the final blow to preformation.

In the 1950s, Gurdon carried out nuclear transplantation experiments in which nuclei from differentiated frog cells were transferred to anucleate egg cells. A percentage of the transplanted eggs developed into viable tadpoles, showing that

the master plan for development must be contained within the nucleus. This result and subsequent experiments have led to two major conclusions: (1) that differential gene expression controls differentiation, and (2) that gene expression must be subject to extranuclear control.

Observational studies with the polytene chromosomes of *Drosophila* first led to the concept of genetic programs. The formation of chromosomal "puffs" during early pupation, attributable to RNA synthesis from temporally regulated genes, was studied. Several major conclusions resulted from this work: (1) for any given tissue, puffing occurred at a reproducible time during development; (2) the puffing pattern was tissue specific, and each tissue exhibited its characteristic pattern; (3) the puffing patterns during pupation were reversibly controlled by two antagonistic hormones, the pupation hormone (ecdysone) and the juvenile hormone. Administration of ecdysone gave rise to pupation puffs, while administration of the juvenile hormone gave rise to larval puffs. Thus, hormonal control of reversible gene expression was demonstrated at this stage in the developmental process.

Two possibilities were considered: Independently regulated gene products could control sequential gene expression, or there might be an obligatory sequence of genetic activation steps, such as

$$a \rightarrow b \rightarrow c \rightarrow d \rightarrow e \rightarrow f \ldots$$

In order to test these hypotheses, portions of the *Drosophila* chromosome were deleted (i.e., genes *b*, *c*, and *d*) leaving genes *a*, *e*, and *f* intact. The temporal regulation of the remaining genes was not altered. This observation favored the suggestion that transcriptional regulatory proteins controlled the temporal expression of at least some structural genes during development.

Studies with *Drosophila* also led to the realization that the expression of other genes led to irreversible developmental decisions. Further, expression of reversibly controlled genes may depend on the expression of an irreversibly controlled event. For example, once the bithorax gene is activated, the genes in the metathoracic segment are switched into a new pathway that produces wings. All "wing" genes are activated sequentially. It is now clear that gene activation can occur either reversibly or irreversibly, depending on the specific gene under study, and that the *irreversible* events represent important decision-making steps (*commitments*) occurring during embryogenesis.

Biochemical programs, clearly the direct result of the expression of genetic programs, have been characterized. Thus, synthesis of histone types during sea urchin development is temporally regulated (Figure 1.1). Some histones are synthesized early, others late in development, and none of the late-appearing histones are apparently derived from the earlier ones. The temporal sequence evidently involves activation and silencing of histone genes during specific stages in development.

Biologists concerned with developmental problems in biology have usually approached their systems from one of several standpoints. First, they have characterized the sequence of events that comprises a cyclic or linear progression

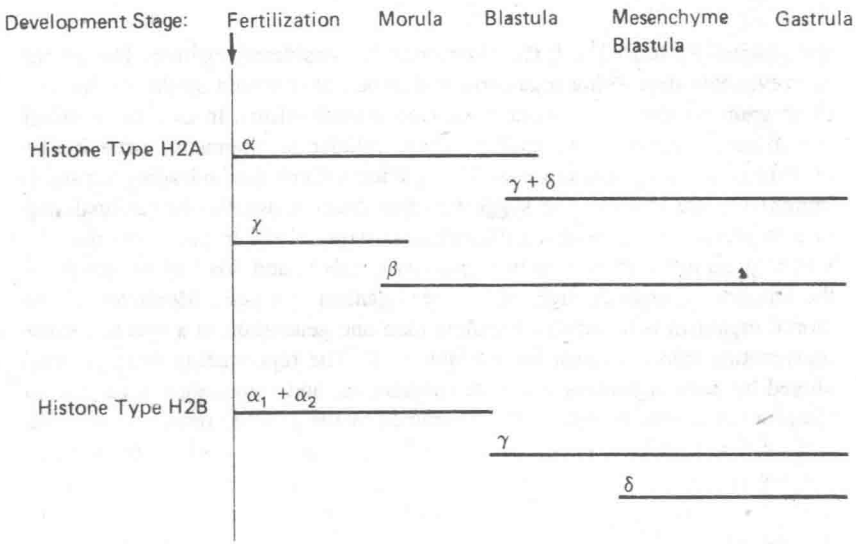

Figure 1.1. Temporal patterns of histone synthesis during development of a sea urchin embryo following fertilization. Two histone classes, types H2A and H2B are shown. The Greek letters refer to the histone subtype. (After L.H. Cohen, K.M. Newrock, and A. Zweidler, *Science 190*:994-997 (1975).)

using classical observational techniques or biochemical approaches. Second, they have examined the genetic mechanisms that allow expression of differentiation-specific functions. These genetic mechanisms can be divided into two classes: (1) those mechanisms which activate silent genes to a state in which expression becomes possible, and (2) those mechanisms that allow expression of an activated gene. Some of the former events may prove to be due to changes in the state of DNA methylation or to genetic rearrangements that render a gene *potentially* active (see Chapter 7). The latter events may correspond to regulatory interactions of proteins or other biological macromolecules with the nucleic acid resulting in enhanced rates of transcription and translation. Both genetic activation mechanisms may be required for expression of a particular differentiation-specific trait. Third, biochemists have examined individual processes peculiar to a differentiated cell in order to understand the mechanisms by which the aforementioned gene products function. Examples include mechanisms of cell–cell interaction (Chapter 10) and ligand–receptor interaction (Chapters 6–9), and these interactions may in turn influence gene expression. Some of these interactions may function to sensitize the genetic apparatus to concentrations of small molecules in the cytoplasm or extracellular matrix. Thus, feedback and feedforward regulatory mechanisms in gene control during development can be expected to be mediated by macromolecular receptors in the cytoplasm and plasma membrane.

In this volume we shall be concerned largely with three phenomena: Differentiation, Sex, and Death. It can be argued that these three topics are intimately

and obligatorily related and, therefore, must be considered together. The advent of irreversibly developing organisms had to be accompanied by the appearance of programmed death and sexual reproductive mechanisms. In fact, the terminal step in any sequence of irreversible steps of cellular development must either be death or immortality, and few examples of linear differentiation leading to natural immortality are known. The suggestion that death is usually the terminal step in a sequence of irreversible differentiation steps results in part from the fact that no organism carries an infinite quantity of DNA, and this fact renders finite the number of steps through which the organism can pass. Moreover, if the mortal organism is to survive for more than one generation as a species, some rejuvenating principle must be available to it. The rejuvenating principle employed by most organisms is sexual conjugation, and conjugation gives rise to offspring of genetic compositions determined by the genetic complements of the parents. The biological phenomena of Differentiation, Sex, and Death are inextricably linked in the existence of any mortal organism.

In order to avoid ambiguity and to progress toward an understanding of biological development, a number of terms must be defined. *Evolution* is a progressive process that can give rise to a present day adult organism. One can consider *phylogenetic evolution*, in which case one refers to the sequential events that occurred throughout phylogeny, i.e., over biological evolutionary history on earth (about 3 billion years), during which the organism developed increasing degrees of complexity. Alternatively, we may consider *ontogenetic evolution*, referring to the development of a single cell or organism through ontogeny, from a fertilized egg to the last irreversible differentiative step.

Differentiation can be defined as the development of function, while *morphogenesis* refers to the development of shape. *Development* encompasses both differentiation and morphogenesis. It is perhaps best defined as "genetically directed changes in a single cell, or groups of cells, assumed in a programmed fashion over time."

Some organisms undergo irreversible but *cyclic development*, while others are only capable of *linear development*. For example, certain bacteria and lower eukaryotes such as yeast differentiate into resting spores which subsequently germinate into the vegetative organism. These organisms are said to cycle through development. By contrast, all "mortal" multicellular organisms pass through a linear program of differentiation, never cycling back to an earlier state. In order for such an organism to become *rejuvenated*, it must undergo *sexual conjugation* during which genetic material is transferred between organisms of a single species, giving rise to a new, genetically unique individual.

Death can be considered to be the cessation of life, but *life* is not so easily defined. We can compare *genetic*, *organismal*, and *cellular death*. A living organism that is incapable of reproduction is said to be genetically dead. By contrast, an animal that lacks a heartbeat and no longer emits brain waves is organismally dead, even though individual cells within its body may live on. Moreover, we can distinguish *accidental death* from *programmed death*. Accidental death refers to the loss of life as a result of environmental factors external

to the organism itself. By contrast, programmed death refers to a terminal step in a program of irreversible differentiation that results in loss of life. Extending this argument one step further, we shall define a *mortal cell* or organism as one which is programmed to die while an *immortal cell* is one which can live forever, provided that external conditions remain favorable. By this definition, simple bacteria and germ cells of higher organisms must be considered to be immortal because they possess the potential to live forever. Unicellular ciliates, such as Paramecia, and somatic cells of higher organisms, on the other hand, are considered to be mortal because they are destined (programmed) to die. Only genetic changes that disrupt a developmental program can rescue an irreversibly differentiating cell from the ultimate fate of mortality.

Finally, what is meant by the term *microorganism*? This term has been defined, on structural grounds, as an organism which exists in the unicellular state; on functional grounds, as one which is easily subjected to physiological and genetic manipulation, and quantitatively, as one which is small. All of these definitions are arbitrary. Many microbes of both prokaryotic and eukaryotic cell structure can exist either in a unicellular or multicellular state depending on the stage of their life cycle. Technical definitions are difficult as techniques for the genetic and physiological manipulation of higher organisms are constantly becoming more refined. And most organisms would be considered small relative to an elephant or a redwood tree. For the purpose of this discussion, we shall define a *microorganism* as one which can be subjected to the experimental techniques of the microbiologist and can be reduced to the unicellular state for examination by the cell biologist. All the organisms to be discussed in this volume fall within this category.

Comparative studies of prokaryotic and eukaryotic microorganisms led to the suggestion that genetic mechanisms responsible for the various modes of differentiation may have evolved prior to the advent of nucleated cells. Thus, the numerous and striking similarities in developmental processes occurring in select members of the prokaryotic and eukaryotic worlds suggest that in divergent organisms, evolution has occurred with retention of a particular mode of development. A second possibility is that convergent evolution of developmental processes has occurred in divergent species, as a result of a common environment and similar evolutionary pressure. It should be pointed out that divergent and convergent evolution are not mutually exclusive, but could have occurred simultaneously. Common environmental pressures would be expected to promote the expression and utilization of shared genetic material of value within a particular environmental framework. Additionally, genetic exchange between evolving prokaryotes and eukaryotes undoubtedly occurred, and such exchange would be most likely between organisms sharing a common habitat. Numerous examples of amino acid sequence homology in proteins of eukaryotic and prokaryotic origin support these contentions. The thesis that convergent and divergent evolution represent components of a single process will be reiterated and discussed in Chapter 4. In the remainder of this chapter, several examples of superficial similarities between prokaryotes and eukaryotes will be discussed. Common

molecular mechanisms and developmental programs will undoubtedly be shown to underlie some but not all of these similarities.

Certain bacteria, like animal cells, can alter their shape and interact with each other by recognition processes that must involve cell surface homotypic or heterotypic adhesive macromolecules (see Chapter 10). *Ancalomicrobium adetum* is a bacterium with a flexible star-shaped appearance that exhibits adhesive self-recognition properties, allowing for intimate contact. *Myxobacteria* also show different responses when encountering bacteria of the same and different species. Thousands of myxobacteria migrate in cohesive packs seeking live prokaryotic food sources which they lyse and devour. *Bdellovibrio,* on the other hand, is a small bacterium which does not adhere to like cells but will bind tenaciously to the outer membranes of certain Gram-negative bacterial species which it can parasitize and kill. Heterotypic adhesion induces the secretion of digestive enzymes which locally rupture the outer membrane and peptidoglycan layer of the larger prey bacterium, thus allowing the predator to enter the periplasmic space, between inner and outer membranes. *Bdellovibrio* then reproduces at the expense of host-derived nutrients and eventually lyses the host cell.

Bacillus and *Saccharomyces* represent prokaryotic and eukaryotic genera, respectively, which under unfavorable growth conditions form resistant resting endospores. The sporulation and germination processes are in many respects amazingly similar in the two types of organisms. Members of the *Actinomycetes (Streptomyces, Actinomyces, Nocardia)* form extensive mycelia that so resemble those of fungi that these bacteria were originally classified as fungi until biochemical studies revealed the presence of peptidoglycan-containing cell walls. Members of the *Actinomycetes* also sporulate with the formation of conidiospores in a process not unlike conidiation in *Neurospora*.

While the majority of both bacterial and nucleated cells reproduce asexually by binary fission, yeast and the prokaryote, *Rhodomicrobium vanniellii,* reproduce asexually by budding processes (Figure 1.2). In both species, buds form which lack DNA, and subsequently, after the occurrence of DNA replication in the mother cell, the newly synthesized nucleic acid is transferred to the developing bud. Dual and relatively independent control processes apparently account for the budding and replicative events.

A few bacterial species exhibit spatial as well as temporal control over cellular differentiation. In these species multicellular prokaryotic structures form, and different cells within a population, originally of a single cell type, give rise to different cell types. The type of program to be expressed by any one cell is both temporally and spatially regulated. Examples of such behavior include the blue green bacteria that differentiate into photosynthetic vegetative cells and non-photosynthetic nitrogen-fixing heterocysts (Figure 1.3). These two cell types arise from the vegetative cell in a carefully regulated sequential fashion, and the mechanism proposed for the spatial regulation of heterocyst development is strikingly similar to that proposed for spatial regulation of development in the coelenterate, *Hydra*. It should be noted that several studies have led to the conclusion that heterocyst development in blue green bacteria is irreversible: a