The Role of Chromosomes in Development

Edited by

Michael Locke

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Contributors and Presiding Chairmen

Numbers in parentheses indicate the pages on which the authors' contributions begin.

R. ALEXANDER BRINK, Department of Genetics, University of Wisconsin, Madison, Wisconsin. (183)

Chairman: HERBERT STERN, University of Illinois

HARRIS BUSCH, WESLEY C. STARBUCK, ERIC J. SINGH, and TAE SUK RO, Department of Pharmacology, Baylor University College of Medicine, Houston, Texas. (51)

Chairman: HEINRICH URSPRUNG, The Johns Hopkins University

JAN-ERIK EDSTRÖM, Department of Histology, University of Gothenburg, Gothenburg, Sweden. (137)

Chairman: JOSEPH G. GALL, Yale University

T. C. HSU, WERNER SCHMID,* and ELTON STUBBLEFIELD, Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas. (83)

Chairman: HANS STITCH, Queens University, Ontario, Canada

E. B. LEWIS, Division of Biology, California Institute of Technology, Pasadena, California. (231)

Chairman: DONALD POULSON, Yale University

CLEMENT L. MARKERT, Department of Biology, The Johns Hopkins University, Baltimore, Maryland. (1)

MONTROSE J. MOSES and JAMES R. COLEMAN, Departments of Anatomy and Zoology, Duke University, Durham, North Carolina. (11)

Chairman: CARL P. SWANSON, The Johns Hopkins University.

DAVID L. NANNEY, Department of Zoology, University of Illinois, Urbana, Illinois. (253)

Chairman: PHILIP GRANT, National Science Foundation

WALTER S. PLAUT and DAVID NASH, Department of Zoology, University of Wisconsin, Madison, Wisconsin. (113)

Chairman: DAVID M. PRESCOTT, University of Colorado Medical School

EDWARD REICH, The Rockefeller Institute, New York, New York. (73)

Chairman: CHARLES THOMAS, The Johns Hopkins University

LIANE B. RUSSELL, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. (153)

Chairman: JACK SCHULTZ, The Institute for Cancer Research, Philadelphia, Pennsylvania

* Present address: Kinderspital Zürich, Zürich, Switzerland.

Oscar Schotté

Dedication

It is especially fitting that we should meet at Amherst College upon the invitation of Professor Schotté to discuss the role of the chromosomes in development. One of the first rays of light to be shed upon this question, and still one of the brightest, issued from the results of Oscar Schotté's xenoplastic transplantations conducted in the laboratories of Hans Spemann and Ross Harrison. His experiences in those laboratories and in that of Guyénot came in one of the most exciting periods in the history of embryology. It was an excitement that was to prove contagious, for it has been communicated through Professor Schotté with an effervescent enthusiasm to his brood of scientific children.

Twice a speaker before the Growth Society and twice its host, Dr. Schotté has devoted the major part of his scientific career to the discovery and elucidation of the physiological correlates of regeneration in the amphibian limb. He has uncovered a wave of changing competence in the anuran limb during metamorphosis, and physiologically distinct phases in the regeneration process itself. The actions and interactions of nerves, of stress, and of the hormones of the pituitary and adrenal glands have been illumined by his probing scrutiny.

It is in grateful tribute to Professor Schotté and to his contributions as an investigator, his inspiration as a teacher, and his congenial passion for the study of development and growth that this volume is dedicated to him.

"The Growth Society"

June 1964

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The Role of Chromosomes in Development

CLEMENT L. MARKERT

Department of Biology, The Johns Hopkins University, Baltimore, Maryland

After the recognition at the beginning of this century that the chromosomes were repositories of hereditary potentialities there soon came the realization that they must also be fundamentally involved in embryonic development. Adult structures could scarcely be inherited independently of their embryonic precursors from which they arose by a long process of orderly development. Like the adult, each preceding stage in development represents a fully integrated and functioning individual whose characteristics must ultimately be traceable to the inherited endowment present in the fertilized egg. Analysis of the zygote reveals two distinct kinds of inherited material, the chromosomes and the surrounding protoplasm of the nucleus and cytoplasm. It is important to realize that the continuity of life narrows between generations not to the chromosomes but rather to an exceedingly complex cell, the zygote, containing a vast array of substances free in solution or arranged in complex specific patterns in gels, membranes, fibrils, macromolecular aggregates, and organelles, each rivaling in complexity many simple organisms. Only some viruses are reduced to the bare minimum of a strand of DNA linking one generation with the next. And even these must use the complex machinery of living cells in order to reproduce themselves. All higher organisms retain a complex cell as the minimal link between generations.

Although studies in genetics have long traced inherited characteristics to the genes on the chromosomes, it was initially perplexing to note that during cell division each daughter cell received an apparently identical set of chromosomes, so that later differences between individual cells or between the organs they composed could not be ascribed to differences in gross genetic makeup. Several explanations have been offered for the diversification and specialization of cells occurring during development.

(1) Although the chromosome sets seem identical in each cell of a metazoan, perhaps 5 or 10% of the genes might be destroyed in each cell, enough to account for the differences between cells without being microscopically visible. On this view, cellular differentiation would be based upon a selective loss of parts of the genome, and indeed a few early cytological studies, e.g., of *Ascaris,* seemed to point to such a mechanism. Studies of regeneration, dedifferentiation, and nuclear transplantation, however, have shown that irreparable loss of parts of the genome, even very small parts, cannot be a general explanation for cellular differentiation. (2) A more likely mechanism is suggested by the observation that the cytoplasm of zygotes is typically very heterogeneous, and daughter cells receive qualitatively different aliquots of this cytoplasm during cell division. The differences in this cytoplasm have been advanced as the essential condition for cellular diversification, and indeed they are. Many analyses over several decades have repeatedly demonstrated that the cytoplasmic inheritance of a cell can determine its fate. (3) Another view is to suppose that the chromosomes undergo some programmed change which is unrelated to their protoplasmic milieu and which enables them to specify the constellation of properties characterizing each adult cell. Such chromosomal autonomy is excluded however by the results of experimental transplantation of cells from one area of a developing embryo to another area. When transplanted at a sufficiently early stage, such cells develop characteristics suitable to their new location and quite different from those they would have acquired if not transplanted. Such experiments demonstrate the dependence of differentiation on the cellular environment and clearly reveal the genome of the cell to be in a dependent, responding position rather than to be the autonomous director of the cell's activities.

It seems obvious that any acceptable explanation for cellular differentiation must involve some mechanism by which the function of the genome is, in effect, regulated by the surrounding protoplasmic environment. Our current understanding of molecular biology enables us to recognize several steps from the gene to terminal character, any one of which might be subject to regulation in such a way as to produce the specialized properties of adult cells. These properties commonly stem from the presence in the cells of particular proteins in characteristic relative quantities. The synthesis of a protein might be regulated by controlling the synthesis of ribonucleic acid (RNA) at the level of chromosomal deoxyribonucleic acid (DNA) or by controlling the activity of the various types of RNA involved in the synthesis of protein; the func-

tion of protein as an enzyme, for example, is further subject to a variety of metabolic controls.

For many years the prevailing view was that genes were active all the time in each cell with diversification occurring at some later step. In contemporary molecular terms this would mean that the initial messenger RNA population would be the same in all cells. This we now know is not true. Although regulatory mechanisms may operate at many levels of cellular organization, certainly one of the most important operates at the level of the gene itself. Moreover, these regulatory mechanisms must determine not only which genes are to function but to what degree as well. Most investigations on gene regulation have made use of bacteria or other microorganisms, and much useful information has been obtained, some of it no doubt applicable to higher organisms as well. There are, however, fundamental differences in the biological expectations of gene regulation in bacteria and in metazoa. These differences stem from requirements of immediacy, stability, and durability. The whole bacterium responds to environmental conditions in an immediately adaptive fashion through reversible gene action that displays great sensitivity to transient stimuli. By contrast, gene regulation during the development of higher organisms involves persistent responses to transient stimuli. Genes are turned on that will continue to function for many years in environments quite different from the one that turned the genes on in the first place. Moreover, these genes may be turned on in only a few cells. In all other cells they are turned off throughout the life of the organism.

The regulation of genes in metazoans seems quite different from what we have come to expect in bacteria. This difference in gene behavior is paralleled by and quite possibly attributable to the quite different organization of the genetic material into chromosomes. Although we speak of bacterial "chromosomes," these bear little resemblance to chromosomes of higher organisms. They do have in common the DNA which encodes their genetic potentialities, but bacterial DNA is essentially naked and free in the protoplasm, exposed to the immediate chemical environment, and responsive to fluctuating metabolic conditions. Metazoan DNA, on the other hand, is part of a complex chromosome containing large amounts of several varieties of proteins as well as other less abundant substances. In addition, it is relatively isolated within the nucleus from the larger part of the cell that is outside in the cytoplasm.

Before restricting our attention to the chromosomes it is important

to note that all hereditary potentialities may not reside in the chromosomes. There are two views of the relationship of genes to cell structures as mediated by proteins. One holds that the primary structure of proteins—the linear sequence of amino acids composing them—determines all subsequent properties, secondary, tertiary, and quaternary structure, and through these the physiological activity of the molecules and their assemblage into larger aggregates and cell organelles. Moreover, all other organic molecules are synthesized in the organism through the activity of these proteins. This view, then, relegates the complex organization so evident in living systems to a purely derivative position. Given the genes and the environment in which they can function, the organism will eventually be formed completely. The proper environment for the genes is considered to be the product of the preceding generation of genes; so, in effect, the genes are everything. Supporters of this view give a resounding "yes" to the question paraphrased from Harvey *"Omne vivum E DNA?"*

The alternative view, while acknowledging the central role of DNA as a code for protein, regards the cell as the smallest unit of life and presumes that the cell contains arrangements of parts that cannot be directly or completely derived from the activity of DNA. Specifically, the presence of some of the complex membranes or organelles may be necessary in order that more of the same can be made out of the macromolecules synthesized under the aegis of DNA. Such structures may act as essential templates for the assemblage of their constituents and, once formed, would be self-perpetuating. If so, historical accidents which led to their initial formation would have thenceforth been encoded in the structures themselves rather than in DNA. The best examples have been presented by Sonneborn (1964) in his studies of the ciliary patterns and external organelles, such as the mouth and anus, of *Paramecia.* Mitochondria and plastids are also candidates for self-replicating structures not under the direct control of DNA, although some evidence for the presence of DNA in these structures casts doubt on their autonomy.

Though one may question that the chromosomes are the source of all that is significant in the organism, no one can deny their central role both in governing the finished characteristics of the organism and in specifying the multitude of steps along the way from the fertilized egg. Accordingly, this symposium was organized to focus attention on the structure and function of chromosomes during development.

Despite decades of cytological observations the structure of the chromosome is still poorly understood, although we can feel some confi-

dence concerning our knowledge of the major chemical constituents. In the introductory paper of the symposium Moses and Coleman summarized the evidence on the comparative morphology of chromosomes particularly as revealed by the electron microscope. A morphological common denominator of chromosomes is a microfibril about 100 Å in diameter. This fibril is composed of DNA and protein. The role of the DNA is clear, but the function of the protein component is still not resolved. Histones, rich in lysine and aginine, are always present on the DNA and have been the most extensively studied of the chromosomal proteins—because of ease of isolation and characterization and because of the expectation that they may play a key role in regulating the function of DNA. It seems clear that histones of one variety or another can inhibit DNA-dependent RNA synthesis, but this capacity does not make them gene regulators during development. In fact, genes appear to be inactive during early cleavage stages and only become active later in development after the appearance of acidic proteins on the chromosomes (cf. chapter by Busch *et al.*). This suggests that the histone inhibition of DNA heterosynthetic activities may be removed through the activity of a more acidic type protein; but these proteins have been little studied and, consequently, there are few constraints on speculation.

The work of Edstrom clearly demonstrates that inactive genes cannot be due to the presence of RNA, for only a small minority of the bands on dipteran salivary gland chromosomes contain any RNA and these appear to be the active regions. Furthermore, RNA is absent from the chromosomes of sperm, that must be almost completely inactive. When RNA does appear, it probably is one of the three kinds of recognized RNA—mRNA, tRNA, or rRNA—any one of which would indicate the activation of DNA rather than its inhibition. Moreover, since RNA is an immediate product of gene function, it does not seem to be a logical contender for the role of gene activator. Both the repression and derepression of gene function appear to involve proteins—probably histones to repress and acidic proteins to derepress or activate.

The nature of the combination between DNA and various kinds of protein is not clear, although it seems probable that the histones combine through salt linkages between their arginine and lysine amino groups and the phosphate groups of DNA. Little is known of the combination between acidic proteins and DNA and essentially nothing as to what might underlie the apparent specificity of such combinations. However, the work of Markert and Ursprung (1963), Ursprung and Markert (1963), and Kimmel (1964) on the effects of proteins injected into

frog eggs demonstrates some affinity between these nonhistone proteins and the chromosomes. In their experiments abnormal combinations presumably occurred between injected protein and DNA leading to failure of normal replication together with the production of breaks in the chromosomes. In any event, the chromosomes were not able to fulfill their normal roles at the time of gastrulation as shown by the cessation of further embryonic development. Several lines of evidence point to gastrulation as the time in embryonic life when genes first become active and essential for further development. Any interference with the normal programming of gene function at that time would probably lead to serious abnormalities or, as in the cited experiments, to complete cessation of development. Partly in an effort to gain insight into the combining properties of DNA with protein, Reich has carried out an extensive examination of the combination of actinomycin with DNA. This peptide interferes with the synthesis of mRNA through an inhibitory combination with DNA. Unfortunately, one of the limitations in applying an analysis of actinomycin behavior to gene regulation is that it inhibits mRNA synthesis. What is needed is a molecule that will stimulate RNA synthesis, i.e., to release the DNA from its normally inhibited state. Of course, the more we know about the chemical basis of inhibition the more likely we are to gain insight into possible mechanisms of stimulation.

Both Hsu and co-workers and Plaut analyzed the discontinuous labeling of DNA with tritiated thymidine during cell division. They demonstrated that the chromosome is not in a uniform state from one end to the other, nor does it replicate as if it were simply a very large and very long bacterial "chromosome." Rather the chromosome behaves as if it consisted of a longitudinal series of linked DNA molecules, each replicating independently. The replication, however, seems to follow a consistent pattern since the same regions on homologous chromosomes replicate synchronously, thus pointing toward control mechanisms affecting the same genes alike even though on different chromosomes.

Perhaps the most significant point to be gained from these observations is that the physical state of the chromosome may regulate gene function. During the intermitotic period when genes are functioning and RNA is being synthesized (see chapters by Edstrom and Hsu *et al.*) some parts of the chromosomes remain condensed or tightly coiled. These heteropycnotic regions are inactive in transcribing the DNA code to RNA and lag behind in the synthesis of DNA as the time for cell division approaches. Although molecular events must underlie gene regulation,

it is apparent that these events may be implemented by changes in the steric conformation of the chromosome, thus regulating its ability to function as a template both for RNA and for DNA synthesis. The cytologically evident conformational changes in the chromosomes do not permit any estimate as to what fraction of the genome may be inactive at any one time. Several lines of evidence (Hoyer *et al.*, 1964), however, point to the conclusion that only a small part of the genome of any cell is functional at any one time (cf. also Edstrom). Thus the normal state of the gene appears to be one of inactivity, probably because of its combination with histone even though this is not evident as heteropycnosis. Certainly many genes, perhaps nearly all, are inactive in the early cleavage stages of an embryo. Progressive changes in the chromosomal environment eventually turn on certain genes in the proper sequence. These newly functioning genes synthesize products which lead to the activation of additional genes and perhaps also to the inhibition of some of those previously functioning; such a dynamic cyclical interplay between genes and environment is logically adequate as a mechanism for driving the cell along the pathway of differentiation. Once specialized adult status is reached cells generally exhibit great stability in their differentiated characteristics, but this stability may only reflect the constancy of the cellular environment. When removed from their normal environment, as when transplanted to culture, cells commonly lose many of their specialized characteristics, particularly after several division cycles have been completed. Thus the differentiated state of the chromosomes is ultimately dependent upon a stable protoplasmic environment. The degree and immediacy of this dependence must, however, vary widely from one chromosomal region to another. Perhaps the clearest and most conspicuous example is provided by the mammalian X chromosome (see chapter by Russell). In the female embryo, one of the two X chromosomes in most cells soon becomes heteropycnotic and thereafter through succeeding cell generations gives rise only to additional heteropycnotic chromosomes. The conformational state of the chromosome and its descendants remains fixed even though other properties of the cells change enormously. Here the regulatory mechanisms are not directed at each individual gene separately but at large sections of the X chromosome. A wave of inhibition seems to spread along the chromosome, or in the reciprocal view a wave of activation may spread in the opposite direction. Since additional X chromosomes in a cell do not become active and since the initial condition is one of inactivity, the hypothesis most consistent with the general view expressed here makes

the partial activation of one X chromosome the positive event in the cell. Whatever molecular events may be involved, they appear sufficient from a quantitative view to act on only one chromosome; all others remain inactive.

Perhaps most significant is the observation that the control, whether activation or inhibition, acts at the chromosomal rather than at the gene level. Whether a gene functions or not depends upon its location in the X chromosome and not upon its own characteristics. Translocated to an autosome, it functions, or translocated from an autosome to an appropriate part of the X chromosome, it is inhibited from functioning.

In many ways this behavior of the X chromosome resembles variegated position effect. Some change in the chromosome, spreading along it to varying degrees, alters the function of the genes encountered. This change is evident in the heteropycnotic character of the inactive region which involves a physical change in the conformation of the chromosome and possibly also extensive chemical changes. That the functioning of the genes may be affected by adjacent regions of the chromosome may also be illustrated in the paper by Brink on paramutation at the *R* locus in maize. This puzzling phenomenon results from a persistent modification of gene behavior as a consequence of the temporary presence of a chromosome bearing alternate alleles. Although an explanation in molecular terms cannot be provided yet, it is obvious that one region of a chromosome must be influencing another. How far such influences may spread seems to follow no general rule. *Cis-trans* arrangements are important in the bithorax mutants of *Drosophila* studied by Lewis, but many degrees of interdependence between chromosomal regions have been established in studies of different organisms. The bithorax genes seem to act as repressors of alternate systems of cellular differentiation, but this apparent specific repression may only reflect the canalization of development arising out of mutual exclusion in the activation of sets of genes.

A similar all-or-none choice in gene activation is illustrated during nuclear differentiation of ciliates (see chapter by Nanney). The macronuclei of these organisms are composed of many subnuclei, the properties of which become fixed at a precise time in the life of the organism. This fixation involves the activation of one of the alleles in each subnucleus coincident with a repression of the alternate allele.

This genetic behavior during nuclear differentiation emphasizes a basic and common characteristic of cellular differentiation, i.e., its all-or-none character. The cell types represented in a complex metazoan