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Theoretical and Applied Genetics 4

L.I. Korochkin

Gene Interactions in Development

Translated and Edited by
Abraham Grossman

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Preface

Since the Russian edition of this book was published in 1975 many new research works have appeared which have made necessary some additions for the English edition, to reflect progress in molecular developmental genetics. Recent important findings in this field have brought about essential corrections to the concept of genetic regulation of the process of cell differentiation. The discovery of the mosaic structure of a gene prompted the re-evaluation of our considerations about the regulation of gene activity in eukaryotes, and the data about transcriptional events during ontogenesis are of great importance as well.

Formerly it was generally accepted that a derepression of genes was responsible for cell differentiation in the process of development. Recently three important conclusions have been derived (Davidson and Britten, 1979) which help to pose the problem in a new way: 1) Only a small part of single copy sequences of DNA is represented in nuclear RNA of a given type of cell or tissue: 10% to 20% in sea urchin embryos, 11% in rat liver, 4% to 6% in *Drosophila* cell culture, etc. Since only about 10% of single copy sequences represent the structural genes (Davidson and Britten, 1973), transcription of almost the whole set of structural genes occurs. 2) The complexity of the transcripts from single copy sequences is the same in nuclei of cells of various tissues and in the majority of tissues in various stages of development, but polysomal mRNA sets are different. 3) The nuclear RNA includes copy families of moderately repeated DNA specific for various tissues and different stages of development. Thus the differentiating cells are characterized by an accumulation in high concentration in their cytoplasm of a rather limited number of types of specific mRNA, along with the presence in nuclei in significantly lower concentrations of many other types of mRNA which represent copies of the major part of the structural genes. If this is the case the control of the specificity of mRNA sets in various cell and tissue types is carried out at the post-transcriptional level with the involvement of transcripts from copies of the moderately repeated sequences of DNA (Davidson and Britten, 1979). Therefore some authors have supposed that during early embryogenesis all structural genes are active, and later some of them are repressed (Caplan and Ordahl, 1978).

Another important aspect of molecular-genetic events in the process of cell maturation was recently revealed as a result of detailed analysis of the differentiation of antibody-producing cells. From this it was concluded that rearrangement of genetic material can occur in the process of cell differentiation, and that the allelic exclusion takes place at the transcriptional level. It appears that in the process of lymphocyte differentiation V and C genes coding for variable and constant chains of immunoglobulin molecules join together. These genes are located on one chromosome but are separated by a long DNA segment. The beginning of antibody synthesis is accompanied by the excision of this segment so that V and C genes form one transcriptional unit. For instance, Ig heavy chain genetic material consists of at least three non-contiguous germ-line DNA segments – V_H gene segment, J_H gene segment, and associated C_μ and C_α gene segments.

In the process of lymphocyte differentiation these segments are joined together by means of two different types of DNA rearrangement: V–T joining and C_H switching. When the J_H segment is associated with the C_μ segment the lymphocyte produces immunoglobulin M (IgM). C_H switching denotes DNA rearrangement which substitutes the C_α segment for the C_μ segment, and initiates synthesis of α-chain and IgA molecules by the now fully differentiated lymphocyte (Davis et al., 1980).

The evidence favoring this assumption was obtained in experiments with embryonic and differentiated cells. The transcripts of V and C genes were hybridized with various DNA fragments isolated from mice embryos. However, if DNA was isolated from differentiated myeloma tissue (i.e., tissue that produces immunoglobulins very intensely), V and C gene transcripts hybridized with the same clearly defined DNA fraction (Honjo and Kataoka, 1978; Davis et al., 1980, Rabbits et al., 1980). Apparently the mechanism of allelic exclusion involves no joining of V and C genes in one of the homologous chromosomes which henceforth cannot produce Ig mRNA (Honjo and Kataoka, 1978).

Finally numerous data, some of which are presented in this book, indicate that the regulation of gene activity is carried out with the involvement of specific regulatory proteins. However, the fine molecular mechanisms of the action of these proteins are not yet clear, and their elucidation is one of the major aims of developmental genetics. This field of biology, now utilizing methods of molecular biology and genetic engineering, advances so rapidly that each year brings about impressive new results both of theoretic significance and of practical application to medicine and agriculture.

Caplan A, Ordahl C (1978) Science 201:120–130
Davidson E, Britten R (1973) Quart Rev Biol 48:565–613
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Honjo T, Kataoka T (1978) Proc Nat Acad Sci USA 75:2140–2144
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Foreword for Russian Edition

At the present time, the thesis that the development of an organism is the realization of its hereditary information has begun to be evidently trivial. This realization consists in the fact that genetic information described in DNA is initially recorded into the structure of protein molecules and later, at a higher level, is transformed into the properties and behavior of the differentiating cells. At a yet higher level, the hereditary information expresses itself in tissue and organ formation and, as a result, leads to the appearance of the traits of the parental organism.

It seems that in this accelerated hierarchy of various levels of interaction gene interaction is only a partial aspect of the problem and is more a concern of genetics than of developmental biology. In reality, Professor Korochkin's book demonstrates that gene interaction is an essential aspect of the developmental process, or, in other words, the realization of the heredity information occurs only through gene interaction.

It is difficult to imagine constant, direct interaction of genes. It is possible that an effect of the heterochromatin region on closely located genes (position effect) or the regulation of promotor and operator sites during transcription could be attributed to this kind of interaction. In fact, direct gene interaction occurs through compounds, synthesized under the direct control of certain genes and acting on another gene's functions. As yet we know only little about this kind of interaction. Still in question is the nature of the compounds through which direct intergenic interactions could be realized. In the light of certain assumptions these compounds could be a special type of nuclear RNA, if viewed under a different set of assumptions these regulators would also have a protein nature. Thus, the nature of direct gene interaction is still a matter of doubt and a subject of research, although it is an important part of the whole problem.

The merit of this book lies in that the concept of gene interaction is considered in its widest aspect. It includes not only the direct influence of one gene on another, but also the interaction of genetic information at all succeeding levels of realization. For an understanding of the mechanisms involved in erythropoiesis or simply in the synthesis of hemoglobin it is necessary to know whether interaction occurs between the genes controlling the synthesis of the α and β chains, between the mRNA templates, or

between the polypeptide chains themselves. It is also necessary to know the role played by heme and the genes controlling its synthesis. It is necessary to find the factors which initiate the globin genes and to describe the pathways of genetic control of these factors. However, in one of Wainwright's works it was shown that the beginning of hemoglobin translation in early chick embryos is not dependent upon the activation of hemoglobin genes, since globin mRNA is transcribed early. The synthesis of hemoglobin began after the formation of hemin from the precursor delta-amino levulinic acid, which was synthesized with the participation of a specific enzyme. The translation of this protein on subsequent templates proceeds only after the transcription of an alanine tRNA. This is the crucial point of initial synthesis. This example demonstrates how in the regulation of the synthesis of one protein, i.e., hemoglobin, at least three kinds of gene are working together, i.e., interacting. These three genes are: structural globin genes; genes for the synthesis of delta-amino levulinic acid; and genes for the synthesis of alanine tRNA. The complete picture of this regulation is, obviously, more complex and includes the interaction of other genes.

Interactions between genes are realized also at higher levels of organization – on the level of the differentiation of tissues and organs. Our information about the genetic control of these processes is still incomplete. Possible theories in this field are very schematic and, in many respects, speculative.

The formation of the nervous system during embryogenesis is under the control of specific genes since mutations are known which effect particular neural differentiations. The genes which determine the first steps of neurogenesis should begin their function during the induction of the neural plate as a result of contact with the chordomesoderm. It is reasonable to expect that the inducing properties of chordomesodermal cells are also predetermined by specific gene activities and take place during the late blastula – early gastrula stages. It is possible to conclude that the formation of the nervous system in the spinal part of the embryo is due to an interaction of genes by means of embryonic induction. The genes are active in both nerve and chordomesodermal cells. The directed differentiation of some cells into chordomesoderm and others into ectoderm is accomplished through properties of the egg cytoplasm which are also predetermined by the functions of specific gene systems during oogenesis.

The puff activation of polytene chromosomes in the salivary glands of *Diptera* larvas occurs under the control of the steroid hormone ecdysone. The genes which control the formation of steroid metabolic enzymes are supposedly active in the gland cells where this hormone is synthesized. We can consider the regulation of salivary gland functions as a result of the indirect interactions

of specific genes. All the major events belonging to developmental biology could be expressed in terms of interactions between genes; to paraphrase molecular, cellular, tissue, and organ development in terms of the genetic language is reasonable and constructive. Only in this manner is it possible to gain a complete understanding of the pathways of realization of the genetic information in the development of the organism.

To turn from these trivial and obvious propositions to the concrete, well-known mechanisms which are involved in the genetic basis of development is a complicated problem which requires an erudite approach. This book is dedicated to the description of these mechanisms. The author discusses in detail and at a current level the known facts and arranges them in the order which allows for a systematic conceptualization of the problems discussed. This monograph is also unique in that such a summary has not yet been published, although there is a great deal of original data on interactions of genes during development. This is why the appearance of this book in the *Developmental Biology* series is not only useful but also necessary.

At the same time, this book is not merely a simple compilation, devoid of the author's personality. Professor Korochkin's works are well known. In his research he fruitfully combines good genetic analysis with modern biochemical and cytochemical methods of molecular biology. Professor Korochkin's works, with the aid of ultra microchemical methods, which allowed to formulate the original concept of multilevel gene regulation of individual development, are of great interest.

All of these make Professor Korochkin's book both interesting and informative and, in some places, worthy of discussion.

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Part 2 Gene Interaction at the Tissue Level

Introduction

Two general principles in the relationship between genes and characters were already formulated in the 1930's. These principles are: (1) Every gene influences all of the traits of an organism, although its effect on some of them could be small to the point of extinction. (2) Any character of an organism depends on all of the genes in the genome in general, although this dependence of some of them is not noticeable (Astaurov, 1968). This means that the development of each trait is due to a number of successive gene interactions, acting in specific conditions (Rokitsky, 1929).

The dominant–recessive interactions of allelic genes, gene complementation, epistasis, and the modification an interactions of nonallelic genes have been the object of investigations in genetics for a long time.

Dominant–recessive interactions are not always strictly fixed. In certain cases the dominance of one trait over another could depend upon the environment. For instance, the dominant mutation *Abnormal abdomen* in *Drosophila melanogaster* could appear as a recessive as it did when flies were cultivated with an excess of fresh food and under heightened humidity (Morgan, 1924). Moreover, the dominant–recessive nature of two genes could be changed as a result of the position effect of one of them. In 1926, Chetverikov proposed the role of the genotypic environment in the development of traits. He assumed a dependence of gene expression upon their placement in the chromosome and, upon replacement of a gene, a resultant change in the gene expression. Later, the position effect of genes was demonstrated (Sturtevant, 1925; Dubinin and Sidorov, 1934). Obviously there is a biochemical interaction between genes closely associated in a chromosome or between their primary products.

As a result of complementary interactions between dominant genes, it is possible for a new character to develop. This new character differs from the traits determined by each gene separately. For instance, in the formation of cyanide in white clover a complementary interaction is necessary between two genes, which is realized on two levels; the formation of the linamarin substrate and the synthesis of the linamarase enzyme which converts linamarin into cyanide. Each of the processes is controlled monogenically by the nonallelic dominant genes Li and Ac (Atwood and Sullivan, 1943).

The term epistasis designates the suppression of one gene by another, nonallelic, gene (suppressor or inhibitor) which may be either dominant or recessive. Genes may also display a modifying effect in increasing or decreasing the expression of a trait which is under the control of a structural gene.

Finally, in the case of so–called quantitative characters (rate of growth, weight of an animal, and so on) it is convenient to refer to interactions of polygenes. It

is supposed that some traits are dependent upon a number of genes with synonymous effects.

Until recently, the role of gene interactions in the phenotype expression of traits was analyzed basically at the morphological level. Only in some cases, as in the example of complementary interaction cited above, were concrete biochemical mechanisms discovered (see Gvozdev, 1968; Ratner, 1972, 1975).

The current state of developmental biology urgently needs exact descriptions of molecular events which take place in the various types of gene interaction. It is necessary to know how each of these events is involved in gene interactions which produce their effects at different stages of ontogenesis. In other words, "we have to understand how a gene works", how the genotype controls the processes of biosynthesis, cell physiology, metabolism, differentiation, morphogenesis, and the whole complex interacted process of ontogenesis. On the other hand, how does "feedback" work? How do the biosynthetic products, the peculiarities of cytoplasmic differentiation, and the whole developmental system conduct the instruments of the gene orchestra? Or, it may be better stated, how, on the basis of the dynamic interaction between the nucleus and the cytoplasm, does the cybernetic system of regulatory interrelations of ontogenesis realize the hereditary program of development? (Astaurov, 1968, 1974).

The immediate characterization of gene activity is possible only through determination of direct products – the molecules of ribonucleic acid (RNA). But, on the basis of methods currently available, we cannot, with rare exceptions, control the synthesis of individual RNA molecules. In connection with this, indirect methods for exposing separate gene activities are widely used. These methods are based on analysis of the final products controlled by these genes, especially proteins and isozymes (Ursprung et al., 1968). In this case, as in any classical genetic work, trait polymorphism is analyzed with the aid of biochemical methods (electrophoresis, immunoelectrophoresis, etc.) and established (for instance, protein variability for electrophoretic mobility). After this, the following investigations are performed:

1. Genetic determination of the trait – the number of genes controlling its formation.
2. Specificity of various gene actions and the character of interactions among them. In this case it is necessary to take into account the pleiotropic effect of genes, and also the presence of multiple alleles for some of these genes
3. Gene localization in linkage groups.
4. Allelism of homologous genes of different species in interspecies crosses and combinative analysis (Smirnov and Vatti, 1971).

On the basis of observed data, subsequent experiments could be performed for the investigation of additional questions, such as: (a) the time during which genes controlling corresponding biochemical traits began their action; (b) their connection with the particular stages and processes of cell and tissue differentiation; (c) the factors which inhibit and activate these genes, regulating their activity. At the same time, the influence of other genes on the expression of a controlled trait in the phenotype plays a very important role. Figure 1 demonstrates the attempt to summarize the most common principles of gene interactions during enzyme synthesis. It is obvious that a mutation, disturbing the function of gene-controlled syn-

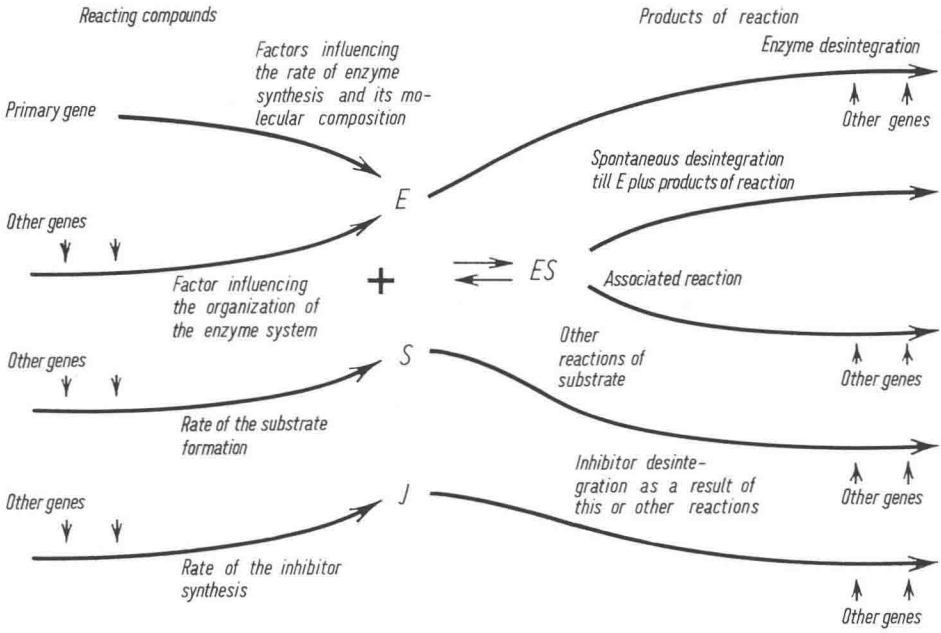

Fig. 1. Some factors of genetic origin influencing the rate of reactions (Wagner and Mitchell, 1958)

thesis of enzyme E, would prevent enzyme activity in the organism and corresponding reactions would not occur, if the organism did not possess another catalyzing agent of a different origin. On the other hand, numerous factors besides enzyme inactivation influence the rate of reaction, from decreasing it to zero to increasing it to a maximum permissible under conditions of the reaction.

Actually, the path from gene to trait is complicated enough, and the process of transcription of a particular gene does not mean that the trait controlled by this gene will be expressed in the phenotype. The events leading to its expression could be realized on the cellular as well as the tissue level, because, in reality, individual development is the processes occurring on these two levels (Olenov,1972).

The cellular level implies the whole complex of processes which are determined by differential gene activity and consist of a system of organospecific syntheses. The latter determines the cell differentiation.

At the same time, it should not be considered, as it sometimes is, that the cellular level of regulation is based mainly upon transcriptional events. In reality, the genetic regulation of biochemical traits on the cellular level could be realized not only through differential transcription, but also through post-transcriptional, translational, and post–translational processes. The relative significance of genetic systems in the control of these processes could differ in each particular case for the total complex of differentiation.

The tissue and intercellular level of trait expression includes complicated intercellular interactions. These relationships are linked with the heterogeneity of differentiated systems and lead to the realization of the corresponding character through

a complex of inductive interactions, intercellular competition, selective proliferation, or the elimination of corresponding cell clones.

The complicated mosaic of interactions between genes, realized at the cellular and tissue levels, finally leads to a specific differential distribution of gene activities in various parts of the developing embryo at different stages of its formation. This is the essence of the genetic control on ontogenesis.

The chapters of this book are dedicated to the analysis of the system of interactions determined by differential gene activity on different levels

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