Genetic Mechanisms of Development

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Edited by

Frank H. Ruddle

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Preface

In this collection of articles, we attempt to survey the application of genetic systems of analysis to the resolution of fundamental problems in developmental biology. The first section deals with the genetic analysis of gene regulation in microorganisms. These systems provide important information in their own right, but in addition serve as paradigms for the genetic analysis of differentiation and morphogenesis in the complex eukaryotes. The second section deals mainly with the application of Mendelian genetics to the study of development in such traditional developmental organisms as *Drosophila,* amphibians, and the laboratory mouse. The third section introduces a new tool for the developmental biologist, *somatic cell genetics.* In these experimental systems, parasexuality in somatic cell populations is used to extract genetic information pertinent to developmental control mechanisms. In a sense, this approach permits the concepts and methodologies inherent in microbial genetics to be applied directly to the study of differentiated soma of higher eukaryotes explanted *in vitro.*

For the first time at the Wesleyan meeting, the Society has organized a public session designed to explore the relationships in a particular area of scientific research and their implications for society as a whole. This first panel session addressed itself to the social impact of genetic engineering. In order to make this discussion broadly available to the public, a videotape recording was made, and this was subsequently edited to produce a 16-mm movie. We have been gratified by the public's response to this film and the demand for its use by schools and organizations. In order to provide even wider dissemination, we have printed a transcript of the sound tract of the film in this volume.

I should like to take this opportunity to extend my sincere thanks and gratitude to the many talented individuals who worked so enthusiastically for the success of the 31st Symposium and for the publication of this volume. It is impossible to give everyone his due recognition but a few should be singled out. The Society particularly thanks the NSF for the generous financial support which made it possible to bring speakers from the west coast and Europe. The Society extends its appreciation to Wesleyan University and its host committee for extending hospitality to the more than 500

attending members and guests. Dr. Winifred Doane, Chairman of the Social Responsibilities Committee, deserves special recognition for organizing the panel discussion and for producing the film record of its proceedings. I should like to thank the officers of the Society personally for their unstinting assistance and support, Mrs. Mary Jo Murnane for her excellent editorial assistance with the manuscripts, and the session moderators and speakers who contributed so ably to the substance of the Symposium and to this volume.

FRANK H. RUDDLE

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Regulation of Bacteriophage λ Development

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I. INTRODUCTORY COMMENTS

I have the prejudice that phage λ ought to be interesting to developmental biologists because I feel that many aspects of λ growth have elements in common with development in more complex systems. For phage λ, as for other biological systems, a single genome in a single cell exercises a choice of temporal pathways. In the case of phage λ, the choice is limited to two major pathways: lysis or lysogeny. The lytic pathway culminates in lysis of the cell and production of more virus particles; the lysogenic pathway culminates in a repressed viral DNA embedded in the host DNA. The basic questions which we would like to answer about these two pathways for λ are, I believe, the basic questions we would like to answer about any developmental pathway: what are the regulatory elements which catalyze the pathways?; what determines the choice between the possible pathways?

In this article, I will try to present a current picture of regulatory events during the lytic and lysogenic pathways. All of the information covered here is presented in similar vein but in much

more detail in other reviews which I have written about the lytic (Echols, 1971a) and lysogenic pathways (Echols, 1971b, 1972). These reviews also contain information on experimental approaches and their current level of application to the various regulatory problems discussed in this article. For a variety of viewpoints, there are also several other recent review articles available on λ development by different authors (Thomas, 1971; Ptashne, 1971; Eisen and Ptashne, 1971). In this summary article, I have not included experimental data; these may be found in the references noted in the text.

II. STAGES OF PHAGE λ DEVELOPMENT

A brief outline of λ development is presented in Fig. 1. For a λ DNA molecule which enters a nonlysogenic cell, there is a choice of the lytic or lysogenic pathway. For the lytic pathway, there is initially a period of synthesis of certain "early" proteins which are involved in DNA replication, genetic recombination, and related phenomena; there follows a period of synthesis of "late" proteins, which are involved in phage head and tail formation and cell lysis; finally lysis ensues and phage particles are released. The value of this temporal organization for lytic development is presumably to provide a period in which the energy and resources of the cell are devoted to viral DNA replication, followed by a period in which the energy and resources are concentrated on the formation of mature virus particles.

For the alternative lysogenic pathway, the period of early protein

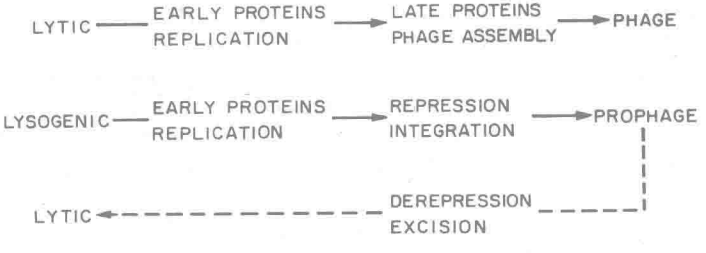

FIG. 1. Brief outline of phage λ development. After a common early stage, the lytic and lysogenic pathways diverge, either to create more phage particles or to produce the repressed, integrated prophage. The developmental process may be started anew either by another phage infection or by derepression of the prophage.

synthesis is followed by a repression of the viral genes and an integration of the viral DNA into the host DNA through a site-specific recombination event. Once established, this "prophage state" is quite stable under normal growth conditions. However, reversal can occur through a derepression of the viral genome, excision of the phage DNA from the host DNA, and lytic development as for an infected nonlysogenic cell. Thus the lysogenic pathway has three stages. The period after infection until the establishment of the prophage state is termed the "establishment" of lysogeny, the stable prophage state and its subsequent inheritance is termed the "maintenance" of lysogeny, and the reversal of this process is termed "induction." The temporal organization of the lytic pathway noted above also serves the multiple needs of the lysogenic pathway, for it allows an efficient consummation of each stage of the lysogenic pathway under the appropriate conditions. This aspect will be considered in Section IV.

III. THE LYTIC PATHWAY

The regulatory problem of the lytic pathway is the temporal organization into a replication-oriented early phase and an encapsulation-oriented late phase. Our current picture of the major regulatory events during the lytic pathway is presented in Fig. 2. The horizontal line denotes a λ DNA molecule. The λ genes are shown in the main generically along the line—clusters of genes involved in head structure, tail structure, genetic recombination, regulatory events, DNA synthesis, and lysis. During the lytic pathway there are three definable stages. The regions of λ DNA transcribed during these stages are indicated by the horizontal arrows. The first or "immediate-early" stage is carried out by the host RNA polymerase; it involves the very limited transcription of the λ DNA represented by the wiggly arrows on the figure (Skalka *et al.*, 1967; Taylor *et al.*, 1967; Kourilsky *et al.*, 1968). Most of the initial RNA synthesis immediately after λ infection probably represents the single gene *N*. In addition, there is some RNA synthesis in the opposite direction ("rightward") from a region of DNA which includes the genes for DNA replication. Following this immediate-early stage, the *N* protein—the product of the *N* gene—activates the "delayed-early" stage in which "leftward" transcription extends through the recombination region and rightward transcription is enhanced from the replication region and extends through

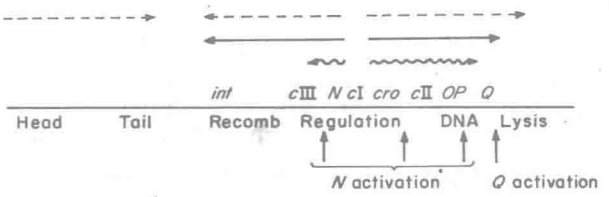

FIG. 2. Transcription events during lytic development by phage λ. Approximate DNA regions transcribed during the different stages of lytic growth are shown: (∿∿∿►) represents the immediate-early stage of RNA synthesis, performed solely by the host transcription machinery; (———►) represents the delayed-early stage of RNA synthesis, in which *N* protein activates transcription of the *c*III to *int* and *c*II to *Q* regions; (– – –►) represents the late stage of RNA synthesis, in which *Q* protein activates transcription of the lysis, head, and tail regions. Since λ DNA exists in a circular or concatemeric form during much of its intracellular life, it is likely that the actual unit of transcription is the circular DNA with the lysis region joined to the head region, rather than the linear molecule extracted from phage and indicated here. The probable sites at which *N*- and *Q*-activation occur are indicated by the vertical arrows (↑). The genetic organization of the λ DNA molecule is indicated by the generic designation below the λ DNA. Specific genes of the "regulation region"—*c*III, *N*, *c*I, *cro*, *c*II—are indicated above the "λ DNA," as are the integrative recombination gene *int*, the DNA replication genes *OP*, and the late regulatory gene *Q*.

gene *Q* (solid arrow on the figure) (Thomas, 1966; Skalka *et al.*, 1967; Taylor *et al.*, 1967; Kourilsky *et al.*, 1968; Radding and Echols, 1968; Kumar *et al.*, 1969; Heinemann and Spiegelman, 1970a).

In turn the *Q* protein—the product of the *Q* gene—activates the "late" stage in which transcription extends through the lysis genes and head and tail genes (dotted arrow on figure) (Dove, 1966; Skalka *et al.*, 1967; Oda *et al.*, 1969). Thus lytic development by λ involves a series of sequential events for which the *N* and *Q* proteins are the essential regulatory elements, exerting positive regulation on other genes.

The biochemical mechanisms for *N* and *Q* activity are not yet known; their understanding awaits an *in vitro* analysis in which the components of the RNA synthesis reaction can be separated and analyzed. Possible mechanisms have been discussed in detail previously (Echols, 1971a).

In addition to this positive regulation, the late stage of lytic development is accompanied by a pronounced repression of early gene transcription, mediated by the *cro* gene product (Radding,

1964; Eisen *et al.*, 1966, 1970; Pero, 1970, 1971; Kourilsky *et al.*, 1970; Franklin, 1971; Court and Campbell, 1972). The physiological role for this turnoff of early genes is not yet clear. The *cro* activity serves an obvious conservation function—to ensure that a cell destined for lysis will not continue to devote energy and resources on unneeded early proteins. In addition, some early proteins might be deleterious to late development. Besides a role in regulation of the lytic pathway, the *cro* product might be important in the choice of lytic or lysogenic pathway. This possibility is discussed in Section IV.

IV. THE LYSOGENIC PATHWAY

A. The Maintenance of Lysogeny

The lysogenic response obviously must involve a turnoff of the lytic pathway. However, the regulatory requirements differ for the establishment and maintenance of lysogeny; not surprisingly, the molecular mechanisms also differ.

The simplest stage is the maintenance of lysogeny. The regulatory problem is the maintenance of an established prophage through a repression of the genes for lytic development and for the reversible integration–excision system. The maintenance of lysogeny is accomplished by a single λ protein, the product of the *c*I gene (Kaiser, 1957; Kaiser and Jacob, 1957). The *c*I protein (or "λ repressor") acts to repress the immediate-early stage of transcription (Isaacs *et al.*, 1965; Thomas, 1966; Pereira da Silva and Jacob, 1967; Ptashne and Hopkins, 1968). This serves to inhibit completely the lytic potential of the virus because of the "prime-mover" status of the *N* protein. If transcription of the *N* gene is blocked, essentially everything else in λ development stops (see Fig. 2). The *c*I protein also acts to provide for its own continued synthesis (Heinemann and Spiegelman, 1970b; Kourilsky *et al.*, 1970; Reichardt and Kaiser, 1971; Echols and Green, 1971).

The repression activity of the *c*I protein is the only aspect of λ development for which the biochemical mechanism has been at all defined because it is the only aspect studied so far *in vitro* with purified components. The *c*I protein binds to operator sites to the left and right of the *c*I gene (Ptashne and Hopkins, 1968) and inhibits the capacity of the host RNA polymerase to transcribe leftward and rightward from the immediate-early promoter sites

(Echols *et al.*, 1968; Chadwick *et al.*, 1970; Wu *et al.*, 1971, 1972; Steinberg and Ptashne, 1971) (see Figs. 2 and 3).

B. The Establishment of Lysogeny

For the establishment of lysogeny, the regulatory problem is more complex than for maintenance because of two considerations: (1) establishment requires not only repression of viral genes, but an efficient integration event; (2) the population of infected cells exercises a choice between lytic and lysogenic responses. The requirement for integrative recombination means that the *int* gene of the recombination region must be efficiently transcribed (Zissler, 1967; Gingery and Echols, 1967; Gottesman and Yarmolinsky, 1968). In addition, integration is probably enhanced by the multiple genome copies provided by DNA replication (Brooks, 1965). Thus, the integration event depends on transcription of the replication and recombination genes. As a consequence, viral development must proceed to the delayed-early stage but stop before an irreversible commitment to lytic growth. The common early step between lytic and lysogenic development probably facilitates physiological regulation of the choice of pathways as well as providing for efficient integrative recombination.

The establishment of repression is accomplished mainly through the action of two proteins, the products of the *c*II and *c*III genes. These proteins probably function in concert at a single site to exert two activities: a turn on of leftward RNA synthesis for the *c*I gene and therefore an activation of synthesis of the maintenance repressor (Echols and Green, 1971; Reichardt and Kaiser, 1971; Spiegelman *et al.*, 1973); an inhibition of rightward transcription from lytic genes and therefore a delay in the onset of the late stage of lytic development (McMacken *et al.*, 1970) (see Fig. 3).

The mechanism by which the *c*II and *c*III proteins carry out their bifunctional regulatory role has not been defined. Their activity probably leads to activation of a new promoter site for leftward transcription (p_E of Fig. 3) and a simultaneous inhibition of rightward delayed-early transcription. This repression in turn can inhibit late gene transcription in two ways; the synthesis of *Q* protein might be insufficient to activate efficiently the late stage of RNA synthesis; the prior stage of RNA synthesis itself might be required to activate fully the promoter sites for the next stage (see Echols, 1972, for a more complete discussion).

The location of the *c*II and *c*III genes and the activity of the *c*II/*c*III proteins provide an effective solution to the regulatory