ADVANCES IN GENETICS

VOLUME 27

# Genetic Regulatory Hierarchies in Development

*Edited by*

THEODORE R. F. WRIGHT

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# Genetic Regulatory Hierarchies in Development

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# PREFACE

In the last five to ten years, the analysis of the genetic regulation of development has made almost unbelievable progress at the genetic, molecular, and cellular levels. The initial supposition that genes in development would be regulated by activator or repressor proteins binding to specific sequences in cis-acting elements has been extensively substantiated. Garcia-Bellido's [(1977) *Am. Zool.* **17,** 613–629] hypothesis that the regulation of gene activity in development would be hierarchical, with "activator" genes regulating "selector" genes (the homeotic loci in *Drosophila*), which in turn would regulate "realisator" genes, has also been extensively confirmed and extended. So much information on the combinatorial and hierarchical control of genes in development has been accumulated recently in numerous diverse organisms that it would be impossible to cover it all in any detail in a volume such as this one. Instead, this volume presents a small, representative sample of genetic regulatory systems in sufficient detail to permit one to appreciate the genetic, cellular, and molecular approaches that have been used to elucidate gene interactions in development and to permit one to comprehend both the complexity and simplicity of these genetic combinatorial and hierarchical networks.

Many of the exciting advances in our knowledge of the genetic regulation of development have been made in *Drosophila,* and the bulk of this volume, six articles, is devoted to this organism. However, since more than 70 genes are now known to be involved in the establishment of polarity and segmentation in *Drosophila,* no pretense is made at complete coverage of this area of research in this organism.

It is hoped that inclusion of articles on *Caulobacter,* yeast, and *Caenorhabditis elegans* will emphasize that other genetic developmental systems have many advantages and much information to contribute to our ideas on the genetic regulation of development. Since no mammalian systems are included in this volume, the reader is referred to the minireview by Blau [(1988) *Cell* **53,** 673–674] for an entry into hierarchies of regulatory genes in mammalian development.

Finally, one might suggest that the investigation of gene regulation in development has been the easy part, in comparison to the now more

difficult task of determining exactly what the protein products of the regulated "realisator" genes actually do at the cellular and molecular level to effect the coherent differentiation and development of different cell types, tissues, and organs.

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# GENETIC REGULATORY HIERARCHY IN *Caulobacter* DEVELOPMENT

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## I. Introduction

Regulatory hierarchies orchestrate the expression of large numbers of genes involved in developmental programs. Eukaryotes and prokaryotes both use cascades of positive and negative trans-acting factors to control the level of expression of many genes in an ordered fashion, for example, mating type expression in yeast, vulva development in the nematode, and formation of the bithorax complex in *Drosophila*. Regu-

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latory hierarchies also control sporulation in *Bacillus subtilis*, fruiting body formation in *Myxococcus xanthus*, and flagellar biogenesis in *Caulobacter crescentus* and *Escherichia coli*. Here we describe the *Caulobacter* regulatory cascade that controls flagellar morphogenesis and then compare the observed mechanisms to those that appear in other prokaryotic developmental systems.

## II. Life Cycle of *Caulobacter crescentus*

Caulobacters are dimorphic, gram-negative bacteria which are found in fresh water, salt water, and soils (Poindexter, 1981). This article focuses on the freshwater *Caulobacter crescentus*, which is the best known species of the group (Shapiro, 1985; Newton, 1989). The organism spends part of its cell cycle as a nonmotile stalked cell and part as a motile swarmer cell. The life cycle of *Caulobacter crescentus* is shown in Fig. 1. The predivisional cell is asymmetric: at one pole is a stalk, an appendage consisting in *C. crescentus* of cell wall and membranes, periodically interrupted by disklike crossbands (Poindexter, 1964). At

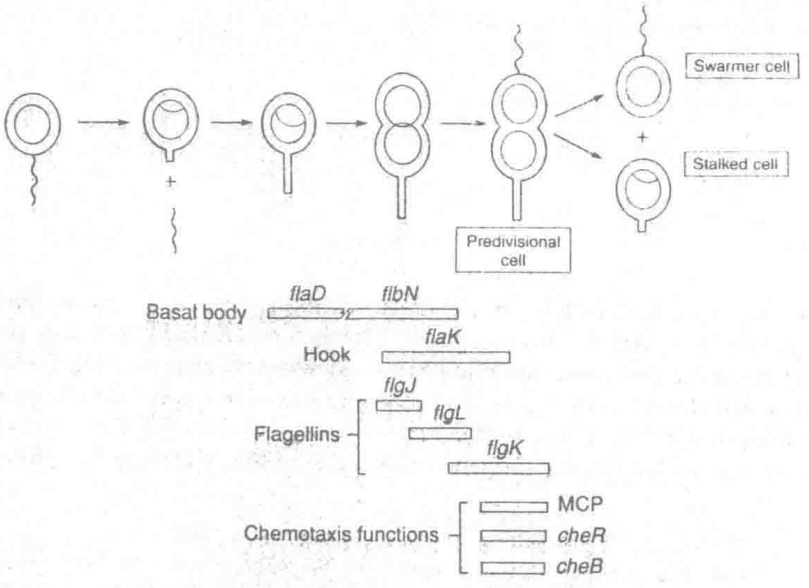


FIG. 1. *Caulobacter* cell cycle. Morphological changes during the cycle are indicated, as well as the timing of expression of flagellar and chemotaxis genes.

the other pole is a single flagellum. Upon division, two different cell types are produced, one flagellated and one stalked, as shown in Fig. 2.

Immediately following division, the daughter stalked cell initiates DNA replication. As it replicates its DNA, it elongates and, in a defined order, produces the proteins needed to build the flagellum at the pole

FIG. 2. Electron micrograph of *C. crescentus* swarmer and stalked cells, negatively stained with uranyl acetate. The helical filament on the swarmer cell is approximately 15 nm in diameter. The stalk diameter is about 90 nm.

opposite the stalk. Following completion of DNA replication and the synthesis and assembly of the flagellum at the swarmer pole, the cell divides. The cell begins swimming just prior to the time of cell division.

After cell division, the daughter swarmer cell does not initiate DNA synthesis. It continues to synthesize the 25K flagellin for assembly of the distal portion of the flagellar filament. After one-third of the cell cycle the swarmer cell loses its filament and begins to grow a stalk at the site where the filament had been attached. At this time, the cell initiates DNA replication, and thereafter the cell cycle continues exactly as in the daughter stalked cell. In rich laboratory media, this cell cycle is invariant, with approximately 30% of the life-span of the swarmer daughter cell spent as a swarmer and the remainder as a stalked and predivisional cell.

Caulobacters are well adapted to survival under conditions of limited nutrients. The stalk, with a large surface area to volume ratio, functions in nutrient uptake. The stalked cell attaches to surfaces using the holdfast at the end of the stalk, while the motile cell (swarmer) can undergo chemotaxis, swimming toward a more favorable location (Shaw *et al.*, 1983). Thus, ecologically, *Caulobacter* exploits two survival strategies each generation: one cell remains and one swims away.

## III. Structure of the Flagellum

The flagellum of *Caulobacter crescentus* is similar to those of *Salmonella typhimurium* and *E. coli* in size and architecture (Fig. 3). It is composed of three sections, the basal body, the hook, and the filament. The basal body, which forms the most proximal portion of the flagellum (Hahnenberger and Shapiro, 1987; Stallmeyer *et al.*, 1989), is composed of five rings threaded on a central rod and embedded in the cell surface, with the innermost ring in the inner membrane and the outermost ring in the outer membrane. One of the middle rings appears to be associated with the peptidoglycan layer. The basal body of *C. crescentus* is quite similar to those of *E. coli* and *S. typhimurium*, except that it contains five instead of four rings. The extra ring is located at the site of filament separation and may be involved in loss of the flagellum each generation (Stallmeyer *et al.*, 1989).

The hook is a helical assembly of 70-kDa monomers (Wagenknect *et al.*, 1981). It is believed that the hook is assembled outside the cell by the addition of protein monomers which travel out through the hollow center of the basal body rod (Macnab, 1987).

The filament in *C. crescentus* is also similar in structure and size to