Current Topics in Developmental Biology

Edited by

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Gerald P. Schatten

Volume 34

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Front cover photograph: The bacterium *C. crescentus*. From Chapter 6 by Roberts *et al.* For details see legend to Fig. 1.

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Preface

The field of developmental biology is fabulous for many reasons, and perhaps foremost among the many strengths of our discipline is its inclusiveness. This volume highlights this aspect: An international group of dynamic scientific contributors explores a range of developing systems wider than those included in the classic dogma, using a wealth of experimental protocols benefitting from molecular, cellular, genetic, and biophysical approaches.

Although developmental biology has typically been viewed as the exclusive purview of eukaryotes, the chapter entitled "Development Programs in Bacteria" by Richard C. Roberts, Christian D. Mohr, and Lucy Shapiro undermines the foundation of this narrow perspective. Determination and differentiation during development are major questions now being addressed most successfully, and the article by Andy Greenfield and Peter Koopman examines the exciting topic of "*SRY* and Mammalian Sex Determination." "Molecular Embryology of Skeletal Myogenesis" by Judith M. Venuti and Peter Cserjesi admirably reviews this lively example of a different type of differentiation.

The molecular mechanisms of fertilization emerge as a subtheme in this otherwise eclectic volume of *Current Topics in Developmental Biology.* The article by Darlene Southworth on the "Gametes and Fertilization in Flowering Plants" reminds us of the wealth of research opportunities in these fascinating systems. Alberto Darszon, Arturo Liévano, and Carmen Beltrán consider what is perhaps the earliest of events during the fertilization process in their chapter "Ion Channels: Key Elements in Gamete Signaling." Because the goal of fertilization is the union of the parental genomes within the activated egg, the emergence of the male pronucleus is a critical event; this is considered by Dominic Poccia and Philippe Collas in their article "Transforming Sperm Nuclei into Male Pronuclei *in Vivo* and *in Vitro*." The precise nature of the parental contributions to the zygote and the embryo is still not completely understood, and the contribution by Timothy L. Karr examines the "Paternal Investment and Intracellular Sperm–Egg Interactions during and Following Fertilization in *Drosophila*."

Together with other volumes in this series, this volume provides a comprehensive survey of major issues at the forefront of modern developmental biology. These chapters should be valuable to researchers exploring development in plant, animal, and now prokaryotic systems, as well as students and other professionals who want an introduction to current topics in cellular, molecular, genetic, and biophysical approaches to developmental biology. This volume in particular will

be essential reading for anyone interested in sex determination, reproduction, fertilization, inheritance, cell-cycle regulation, ionic signaling, embryo formation, morphogenesis, muscle development, and differentiation.

This volume has benefitted from the ongoing cooperation of a team of participants who are jointly responsible for the content and quality of its material. The authors deserve full credit for their success in covering their subjects in depth yet with clarity and for challenging the reader to think about these topics in new ways. We thank the menbers of the Editorial Board for their suggestions of topics and authors. We thank Liana Hartanto, Heather Aronson, and Diana Myers for their exemplary administrative and editorial support. We are also grateful to the scientists who prepared articles for this volume and to their funding agencies for supporting their research.

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1

SRY and Mammalian Sex Determination

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I. Introduction

Two facts feature in any discussion of the genetic basis of sex determination in eutherian mammals. First, the development of an embryo into a male or a female is dependent on whether the bipotential embryonic gonad differentiates into an ovary or a testis. Once the "choice" of the ovarian or testicular pathway of development has been made, all subsequent sexually dimorphic characteristics are the consequence of the hormonal output of the gonads (Jost, 1947). Second, the mammalian Y chromosome is a dominant determinant of maleness (Ford *et al.*, 1959; Jacobs and Strong, 1959; Welshons and Russell, 1959). In normal circumstances, it is the presence or absence of the Y chromosome, not the number of X chromosomes, which determines the sex of the individual. Taken together, these two central tenets allow the geneticist to frame the question of the genetic basis of sex determination in mammals in terms of which gene or genes on the Y chromosome are required for the initiation of testis development. The isolation of the predicted gene(s), known as the testis determining factor (*TDF*)

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in humans and testis-determining Y gene (*Tdy*) in mice, was the subject of an intense international research effort which culminated in 1990 with the identification of the human *SRY* gene (Sinclair *et al.*, 1990). A search through a 35-kb region of the human Y chromosome, the minimum known to be necessary for male sex determination, resulted in the identification of a gene exhibiting all the predicted properties of *TDF*: it was conserved on the Y chromosome of all mammals tested, it was expressed in the developing male gonad prior to overt testis differentiation, and it encoded a DNA-binding protein of obvious regulatory potential. Final proof of the identity of *SRY* and *TDF* came in 1991 in the form of a chromosomally female mouse transgenic for the murine *Sry* gene: this mouse developed as a normal male, albeit sterile due to the presence of two X chromosomes and the absence of Y chromosomal genes required for spermatogenesis (Koopman *et al.*, 1991). *Sry* was thus shown to be the only Y-linked gene (though by no means the only gene) required for testis determination in mammals.

Six years have now passed since the identification of *SRY*. The details of the search for *TDF/Tdy* have been documented elsewhere (Goodfellow *et al.*, 1993). This review will focus on our current understanding of the biology of *SRY* and sex determination. We shall pay particular attention to what is known of the biochemical basis of *SRY* function and its relationship to other genes in the sex determination pathway. Most importantly, we shall attempt to identify those areas in which our ignorance is greatest and address some of the issues which might concern researchers in sex determination over the next 6 years. Data reviewed here will be primarily from studies of mice and humans; the symbol "*Sry*" will be used to refer exclusively to the murine gene and "*SRY*" to that of humans and other mammals.

II. The SRY Gene

A. Gonadogenesis

It is clear from the above introduction that the function of the *SRY* gene is known: its activity results in the development of a testis from the bipotential embryonic gonad. Precisely how this result is achieved, however, is unclear. Before discussing what is known of the structure and expression of the *SRY* gene, we shall briefly review the cellular basis of gonadogenesis in the eutherian mammalian embryo to set the scene for more detailed discussion of *SRY* function.

In the mouse, the gonad has its origins in the genital ridge, a structure which arises as a thickening of the mesonephros at about 10.5 days *post coitum* (dpc). The mesonephros and genital ridge together are known as the urogenital ridge. The developing gonad comprises four known cell lineages common to both males and females: the germ cells and at least three somatic cell types, steroid

cells, supporting cells, and connective tissue. Primordial germ cells migrate from their origin in the primitive streak along the dorsal mesentery and arrive in the genital ridge between 10.5 and 12 dpc. The somatic portion of the genital ridge is derived from mesenchyme and overlying coelomic epithelium, as well as from cells which migrate from the adjacent mesonephros (Buehr *et al.*, 1993; Upadhyay *et al.*, 1981). The supporting cell lineage is the first to exhibit sex-specific differentiation. In males, this lineage produces the Sertoli cells at around 12.5 dpc, while in females it differentiates into the ovarian follicle cells. Alignment of the Sertoli cells results in the formation of characteristic testis cords. The first known product of the Sertoli Cells is Müllerian inhibitory substance (MIS), also known as anti-Müllerian hormone, a glycoprotein which causes regression of the female reproductive tract anlagen. The other primary testicular hormones are testosterone and dihydrotestosterone, which are produced from the male derivatives of the steroid cell precursors, the Leydig cells. These promote the development of the Wolffian duct system into the epididymis, vas deferens, and seminal vesicles. It is the absence of these testicular hormones which results in the "default" ovarian pathway of development. No female counterparts to these hormones, required for development of the female genitalia, have been identified.

If asked to predict, from the above discussion, the timing and sites of *Sry* activity during testis determination and differentiation, one would likely say between 10 and 12 dpc in the developing male gonad. In addition, if we were to predict a precise function for *Sry*, it would likely be in determining the fate of one or more of the gonadal cell lineages discussed above, possibly including the direct activation of genes encoding or regulating the production of the testicular hormones.

B. Timing and Tissue Distribution of *SRY* Transcription

When expression of murine *Sry* was analyzed using the sensitive RT-PCR method, transcripts were detected in adult testis and 11.5 dpc male urogenital ridge samples (Gubbay *et al.*, 1990). More detailed analysis showed that fetal expression is confined to gonadal tissue, does not require the presence of germ cells, and is limited to the period in which testes begin to form (Koopman *et al.*, 1990; Hacker *et al.*, 1995; Jeske *et al.*, 1995). A semiquantitative RT-PCR assay of urogenital ridge RNA samples reveals a profile of *Sry* transcription starting at 10–10.25 dpc, reaching a peak at 11.25–12 dpc, and ceasing by 13.5 dpc (Jeske *et al.*, 1996). This profile shows a striking correspondence with the key events in testis differentiation and the narrow time window in which transcripts can be detected is likely to reflect the importance of the timing of *Sry* expression.

Further evidence that the timing of *Sry* expression is critical comes from the observation that when the *Mus musculus domesticus*-derived *Mus poschiavinus*