Introduction

The current dogma describing the genetic control of development assumes a hierarchy of regulatory genes. In the simplest case, a master control gene directly regulates secondary genes which, in turn, regulate the expression of other genes. In principle the master control genes can be recognized by the pleiotrophic effects caused by mutation, however, complex phenotypic changes are also associated with mutations in many nonregulatory genes. The best-described examples of control genes are from relatively simple organisms with well-developed genetics, for example *Drosophila* and *Caenorhabditis*. Unfortunately, identification of developmental control genes in mammals has proved to be difficult, presumably because homeotic and similar mutations are lethal. There is, however, one well-defined developmental control gene in mammals: *TDF* or the testis-determining gene (the same locus is called *Tdy* in mouse). Molecular cloning of *TDF* will not only facilitate exploration of the fundamental questions of sex determination, but should also provide a model for genetic control of development.

The importance of the mammalian Y chromosome and, by implication, of *TDF* was established by a series of classic observations. First, the presence of the Y chromosomes is irrelevant. Second, the presence of the Y chromosome induces the formation of the testis. Third, secondary male characteristics are due to the production of soluble factors such as testosterone and Müllerian-inhibiting factor by the testis. Overall the process closely resembles an archetypical regulatory network with *TDF*-regulating genes which produce ovaries and testes and the subsequent production of regulatory molecules by the testis. Although the outline is clear many of the molecular details are obscure. The *TDF* locus may include several different genes; the product of *TDF* is undefined and the spatial and temporal expression of *TDF* is unknown. Ignorance of these facts and the absence of a convenient assay precludes simple cloning strategies based on expression. An alternative approach is to exploit chromosomal location. This method has been used successfully to clone several disease loci such as *DMD*, *CGD* and the retinoblastoma gene. The principles involved are simple: define the position of the target gene between flanking marker sequences then clone everything between the markers. The closer the flanking markers the easier to clone the sequences between them. This task is being simplified by the development of long-range cloning and analysis techniques based on pulsed-field gel electrophoresis. Subsequent definition of the target gene depends on techniques such as recognition of open reading frames and cross-species conserved exons.

At first glance, a gene on the Y chromosome is not a good candidate for cloning by exploiting chromosomal position. The major drawback is that most of the Y chromosome does not participate in meiotic exchange and genes on the Y chromosome cannot be localized by Mendelian genetics. However, the Y chromosome is small and translocations have been used to localize *TDF* to the short arm and, as is explained in the following page, a more precise localization of *TDF* can be achieved by exploring the Y-derived sequences present in the genomes of XX males. The stage is set for cloning the mammalian sex-determining gene.

Peter N. Goodfellow
May 1987