<Further Development 6.02>

Finding the Elusive Testis-determining Factor

Finding the male-determining genes has not been easy. One editor (1991) actually wrote, “The search for TDF has been a long and hard one.” The first three editions of this textbook show the incredibly interesting history of sex determination research, as new molecular technologies permitted new types of experiments.

In the first edition of the book (Gilbert 1985), the sex-determining gene was postulated to be a cell membrane factor called the H-Y antigen. It was acknowledged to be a controversial assignment, as its structure was unknown, and it was identified by antisera that female mice made against male cells. It was one of the first factors thought to be encoded by the Y chromosome. Moreover, if ovaries and testes cells were separately dissociated and allowed to re-associate, the testes would form tubules and the ovaries would form follicle-like structures. However, if the H-Y antigen were blocked on the re-aggregating testes cells, the testes structures failed to form, and the cells formed follicle-like structures (Ohno et al 1978, 1979; Zenzes et al 1978). The receptor for the H-Y antigen was found on gonadal cells of both sexes (Nagai et al 1979).

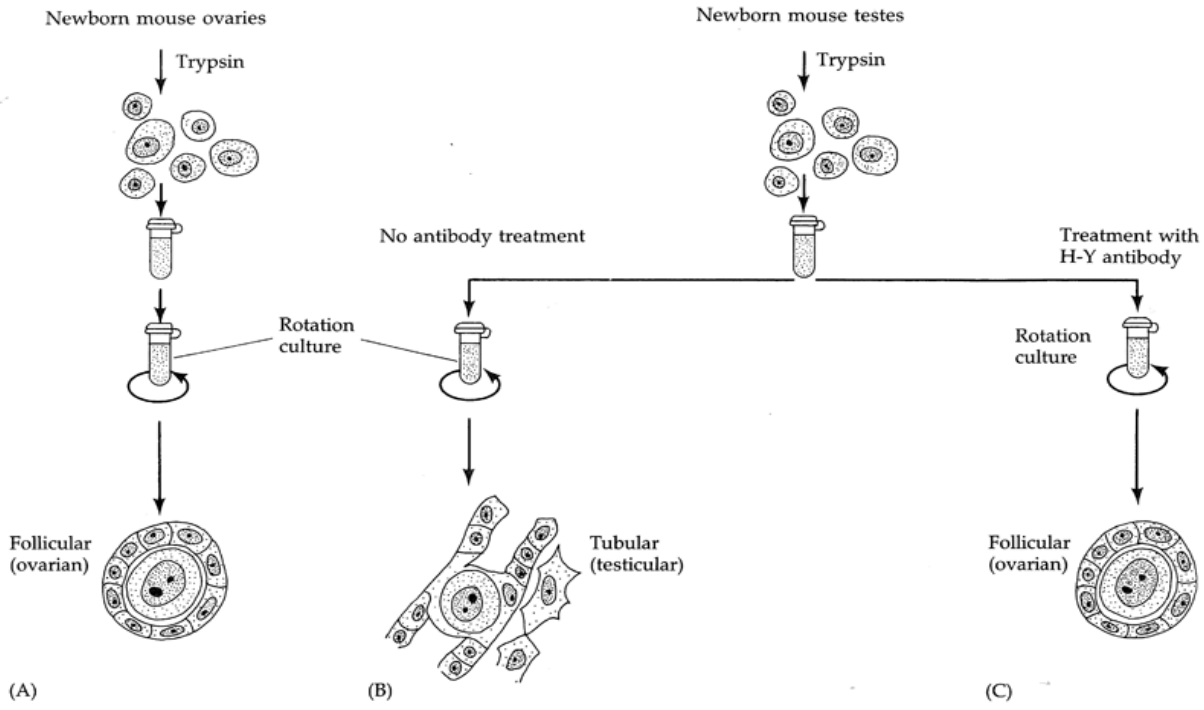

Figure 1 Effects of the H-Y antigen on the re-aggregation of newborn mouse gonadal cells. (A) Trypsinized ovary cells will reaggregate in rotation culture to form follicular-like aggregates reminiscent of ovarian tissue. (B) Trypsinized testis cells reaggregate to form structures that resemble testicular tissue. (C) When the H-Y antigen is removed from the testis cells by antibody treatment, the testicular cells reaggregate into ovary-like structures. (From Gilbert, 1985)

By the second edition of the book (1988), this view had been supplanted by the findings of David Page (1986, 1987) that there was a region of the Y chromosome that was present in XY males, absent in XX females, and missing in XY females, but present in XX males. This DNA region was located at the tip of the short arm of the Y chromosome. Moreover, there was a DNA sequence in this region that appeared to encode for a 404-amino acid zinc-finger transcription factor. This gene was called **ZFY** (Zinc-finger, Y-chromosome).

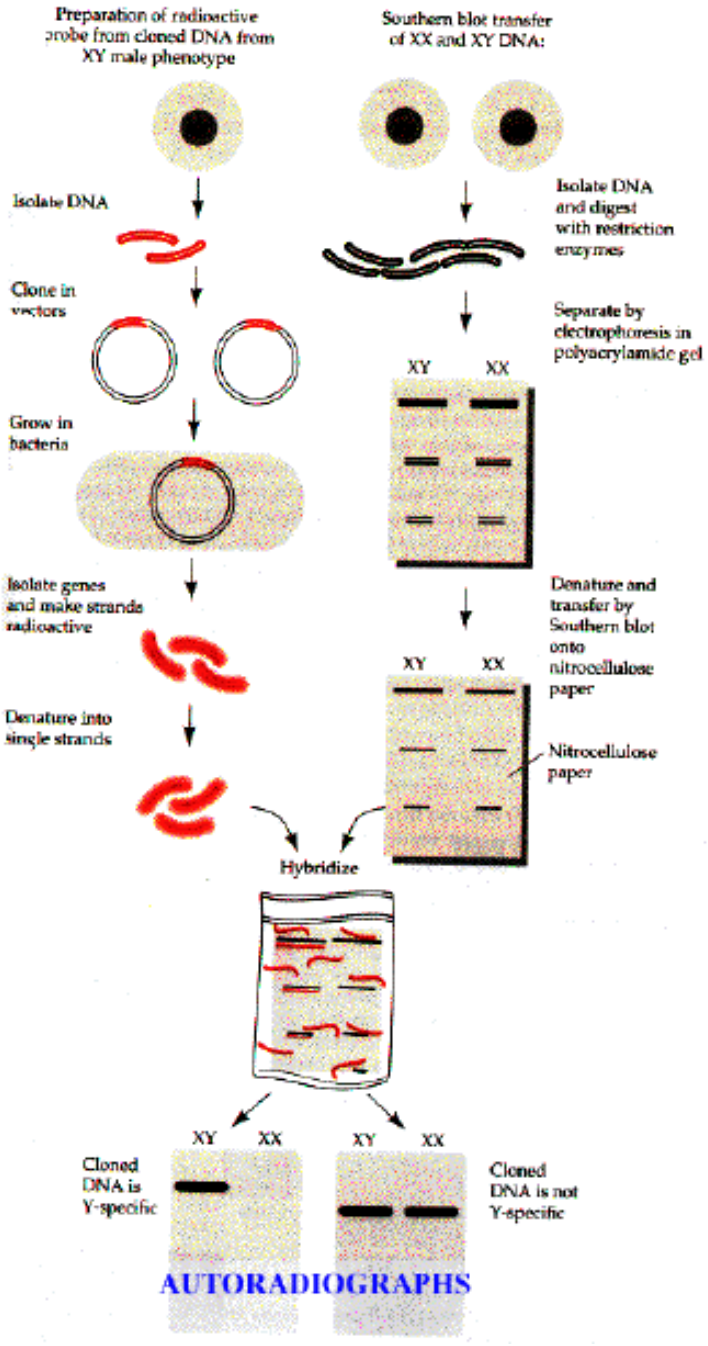

Figure 2 Protocol for screening Y-specific DNA clones. DNA from male cells is cloned into viruses and grown in *E. coli*. Each clone is separately cultured, its human DNA is isolated, and its strands are made radioactive. Each DNA fragment is denatured into single strands and hybridized with DNA from male or female cells that has been

electrophoresed and Southern blotted. The Y-specific DNA fragments should bind specifically to the DNA from XY cells but not to that from XX cells. (From Gilbert 1988).

Page (1988) maintained that until they knew its sequence and function, it was “premature” to call ZFY the testis-determining factor, and noted that the DNA region defined by the translocated chromosomes was big enough for more than one protein-encoding gene (and that his interpretations could be wrong if there were rare double translocations.)

And he was correct. As molecular biology allowed scientists to look at progressively smaller regions, Sinclair and others (1990) showed that the testis-determining factor was not ZFY, but a nearby gene, which was called **SRY** (ex-determining region of the Y chromosome). Anne McLaren (1990) illustrated the progressive winnowing of the genome to find the testis-determining factor.

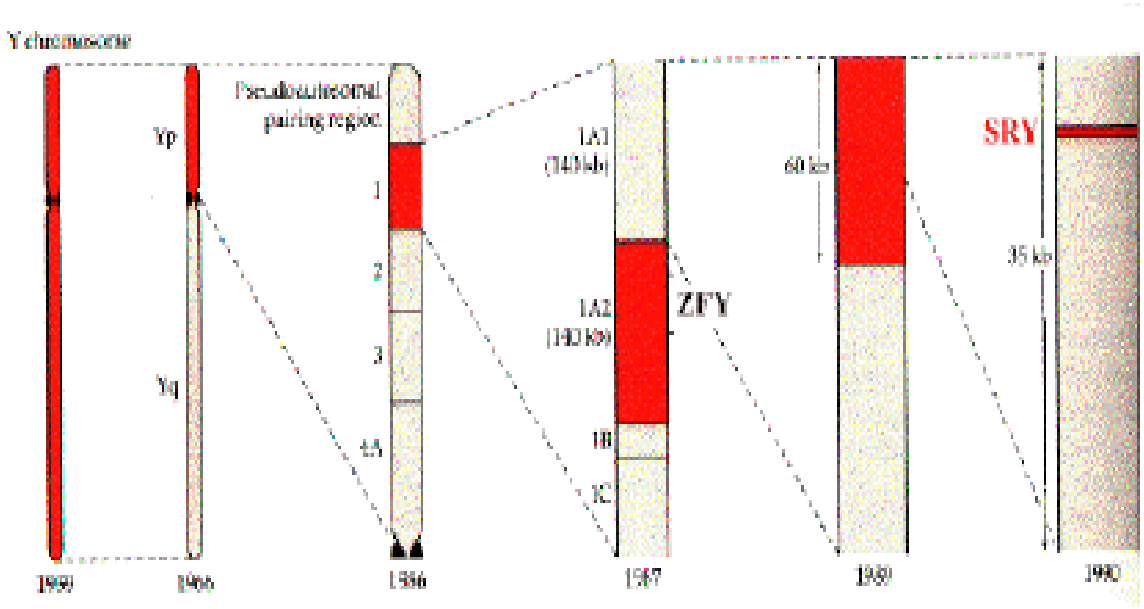

Figure 3 In 1959, the Y chromosome was shown to contain the testis determining factor in both humans and mice. By 1966, translocations and deletions of human chromosomes indicated that the TDF resides in the short arm of the Y chromosome (Yp). In the late 1980s, deletion mapping with male-specific DNA probes showed that the gene(s) resided in region 1 of the small arm of the Y chromosome. Studies of XY females, XX males, and transgenic mice have localized the TDF to a single region on the Y chromosome containing the *SRY* gene. (After McLaren, 1990; From Gilbert 1991).

The author of the textbook heard about the SRY story from Albert de la Chappelle during dinner at a meeting of the Sigrid Juselius Conference in Helsinki in the summer of 1990. The text of the book was just being finished then.

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