"Male Development of Chromosomally Female Mice Transgenic for Sry gene" (1991), by Peter Koopman, et al. [1]

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Early 1990s research conducted by Peter Koopman, John Gubbay, Nigel Vivian, Peter Goodfellow, and Robin Lovell-Badge, showed that chromosomally female (XX) mice embryos can develop as male with the addition of a genetic fragment from the Y chromosome of male mice. The genetic fragment contained a segment of the mouse [4] *Sry* gene, which is analogous to the human SRY gene. The researchers sought to identify *Sry* gene as the gene that produced the testis determining factor protein (Tdf protein in mice or TDF protein in humans [5]), which initiates the formation of testis. Koopman's team published their results in 1991 in "Male Development of Chromosomally Female Mice Transgenic for *Sry* gene." Their results showed that *Sry* gene partly determines the sex of an embryo and is the only gene on the Y chromosome necessary for initiation of male development in mice.

The researchers conducted the *Sry* gene experiment on mice at the Laboratory of Eukaryotic Molecular Genetics, MRC National Institute for Medical Research in London, England, in conjunction with the Human Molecular Genetics Laboratory, Imperial Cancer Research Fund also in London. Koopman and his colleagues had previously worked to identify the gene or genes [6] present on the Y chromosome that partly determine the sex of embryos. Their work had led them to identify a region of the Y chromosome containing the *Sry* gene as a candidate for primary sex determination [7] due to the gene's high degree of similarity among several mammalian species.

Additional support for the claim that *Sry* genes [6] were the primary sex determining gene came from researchers Ralf Jäger, Philippe Berta, and their early 1990s colleagues. The researchers independently published findings from genetic testing of genetically male (XY) humans [5] who developed as females due to either a change in the open reading frame of the gene (frameshift mutation), or a deletion of the region on the Y chromosome containing the SRY gene. The findings provided evidence for SRY gene as a primary sex determining gene, as the loss of function or the absence of the gene in chromosomally male humans [5] resulted in the development of female sex traits in those humans [5]. Furthermore the evidence prompted the MRC team to investigate the *Sry* gene as the only gene necessary in the sexual determination [8] of mice. The team theorized that if *Sry* gene was the primary sex determining gene, then introduction of the gene into chromosomally female embryos (XX) could initiate the development of male sex characteristics in adult mice.

To determine the functional role of the *Sry* gene, the MRC research team introduced a sequence containing the *Sry* gene into mice embryos during early embryonic development. The team used a small genetic fragment from the Y chromosome, which contains about fifty-eight million base pairs. The fragment was fourteen kilobase pairs long and contained the *Sry* gene with small sequences of DNA on either side of the *Sry* gene. The small sequences of DNA contained regions that researchers hypothesized as regulators of *Sry* gene expression during development. Koopman and colleagues obtained the genetic fragments by cutting DNA from the Y chromosome with several enzymes that cut DNA at specific sequences, called restriction endonucleases. The team injected numerous mouse [4] embryos, both male (XY) and female (XX) with the *Sry* gene fragments and implanted the embryos into the reproductive tracts of adult female mice.

The team examined 158 embryos after fourteen days of development for signs of sex reversal from female to male development. First, the researchers looked for the formation of the testis-cord, an early structure in developing male embryos. The presence of the testis cord in embryos signals the sex differentiation [9] has occurred. The team then stained for sex chromatin [10] in amnion [11] cells, which surround the embryos, to determine the chromosomal or genetic makeups of the embryos. The team also analyzed the embryos for presence of the Y-linked gene *Zfy-1* to identify the chromosomal sex of the organisms. Since the *Zfy-1* gene is normally present on the Y chromosome, female mice that developed as males would not contain this gene and therefore must be chromosomally XX.

Of the 158 embryos that the researchers analyzed, most developed as either male containing XY chromosomes or female containing XX chromosome. Two of the embryos were found to be transgenic XX males, or embryos that should have developed as females. These two embryos contained many copies of the *Sry* gene. A histological examination revealed that the testis-cord

formation was normal and indistinguishable from that of normal XY embryos. Furthermore, a total of six XX females contained *Sry* gene in low amounts but developed normally as females. The production of male development in two XX embryos confirmed the claim that *Sry* gene alone could initiate testis development in embryos.

To further investigate the role of *Sry* gene in in the development of sex characteristics, the researchers allowed ninety-three of the embryos containing injected *Sry* gene sequences to fully develop to birth. Five of the animals were confirmed to be transgenic, containing the *Sry* gene from a separate organism. Two of the animals were XY males, and therefore not informative for this study. One of the transgenic mice lacked a chromosome (X0), but it developed male phenotypes such as testes [12] and a penis. The X0 male mouse [4] was similar in size and weight to a normal XY mouse [4]. The X0 male also displayed normal copulation behavior, but due to the lack of a Y chromosome, the mouse [4] could not generate sperm [13] and was sterile. The scientists examined sections of the testes [12] and noted that the testes [12] exhibited normal development of the sexual reproduction tract except that there were no cells making sperm [13]. The observation of male development in the absence of a second sex chromosome further supported the *Sry* gene as the gene that produced the testis determining factor protein (Tdf).

The final two transgenic mice were (XX) females whose DNA sequences contained several copies of the *Sry* gene. To explain this phenomenon the team proposed two theories. One theory stated that the female mice expressed mosaicism for the *Sry* gene, meaning that the *Sry* gene present in the cells of the genital ridge differed from the sequence that was originally inserted, and thus may have lacked the regulatory sequences of the gene. Changes in the *Sry* gene may have occurred through mitotic recombination during development. The researchers' second theory stated that the location of the transgenic *Sry* gene fragment in the embryo differed from that of animals showing sex reversal, and that this difference affected *Sry* gene expression during development. To the scientists, the development of the X0 male, and the male development of genetically female mice embryos, showed that the *Sry* gene was the only gene necessary to initiate the development of male sex characteristics in mice.

Having identified the *Sry* gene as the primary sex determining gene in mice, the team examined the function of the human analog, the SRY gene. Koopman's team injected a twenty five kilobase sequence containing the human SRY gene into mice. The difference in size of the sequence reflected the increased length of the human gene. The team tested whether the nucleotide sequences of the mouse [4] *Sry* gene and the human SRY gene were interchangeable across species. One of the researchers, Gubbay, had previously shown the two genes [6] to be similar, despite the differences of twenty-three of their seventy-nine total amino acids. Koopman and colleagues produced two lines of mice offspring containing the human SRY gene. No transgenic XX females developed as males nor displayed any evidence of altered sexual reproductive tracts. A third line of offspring produced only a single XX transgenic female that again developed normally as a female. The team concluded that the integration of the human SRY gene could not create a sex reversal in genetically female mice, either because it was unable to be transcribed into a protein or it produced non-functional proteins within mice, resulting in normal development in transgenic mice.

"Male Development of Chromosomally Female Mice Transgenic for *Sry* gene"; reports the conclusions of Koopman and colleagues that a fourteen kilobase fragment containing *Sry* gene can initiate testicular formation, and thus sex determination [7] in mice. The data showed that the genetic fragment contained both the entire *Sry* gene and all of the regulatory elements required for its expression in the embryo. The results also suggested that scientists could further analyze genes [6] used in sex determination [7] through the gradual elimination of sequences from the original fragment to determine the function of each sequence. The experiment identified *Sry* gene as a sex determining gene in mice.

Sources

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Subject

Y chromosome [16] Testis [17] Koopman, Peter [18] Embryos [19] Chromosomes [20] Sex chromosomes [21] Sex determination, Genetic [22] Sex differentiation [23] Human chromosomes [24] Genes, sry [25] Sex-Determining Region Y Protein [26]

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