To further investigate the role of the claim that Sry formation was normal 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 and a penis. The X0 male mouse was similar in size and weight to a normal XY mouse. The X0 male also displayed normal copulation behavior, but due to the lack of a Y chromosome, the mouse could not generate sperm and was sterile. The scientists examined sections of the testes and noted that the testes exhibited normal development of the sexual reproduction tract except that there were no cells making sperm. 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 Sry gene and the human SRY gene were interchangeable across species. One of the researchers, Gubbay, had previously shown the two genes 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 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 used in sex determination 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**


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 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), which initiates the formation of testis. Koopman's team published their results in 1991 in Female 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.
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