52. See S. Bashkin and J. O. Stoner, Jr. [*Atomic Energy Levels and Grotrian Diagrams* (American Elsevier, New York, 1975), vol. 1, pp. 388–391] for the energy levels used in this study.
53. A. G. Mitchell and M. W. Zemansky, *Resonance Radiation and Excited Atoms* (Cambridge Univ. Press, London, 1961), pp. 59–70.
54. D. M. Coleman and R. J. Klueppel have photographed the 2795.5-Å and 2802.7-Å lines in the Mg II spectrum in full radial reversal. Klueppel has photographed the 2790.8-Å and 2798.0-Å lines in reversal (Fig. 5) as a function of the repetition rate of the spark source, and the 4481-Å lines at very high intensity. In a separate series of exploratory experiments, Coleman detected the 1734.9-, 1737.6-, 1750.7-, and 1753.5-Å lines photoelectrically. The 2428.6-Å and 2936.5-Å lines have been identified in emission but not yet in absorption. Some positive identifications have been made in the Mg I spectrum, but a full analysis is still in progress. The problem is complex as a result of the wavelength range that must be covered (~1,100 to 12,000 Å).
55. R. J. Sandeman and N. A. Ebrahim, *Appl. Opt.* **16**, 1376 (1977); M. C. E. Huber [in *Progress in Gas Dynamic Research by Optical Methods*, D. S. Dosanjh, Ed. (Plenum, New York, 1971), pp. 85–112] described the "anomalous dispersion" experiment where excited state populations are followed in refracted light. D. M. Coleman is preparing apparatus to do this at Wayne State University. With these instruments it will be possible to carry out interactive experiments with this laboratory for comparative diagnosis of the postdischarge environment with more than one spectroscopic probe [see (*8*) for details].
56. M.-U. Beth, S. Krause, W. Mayerhofer, *Z. Naturforsch. Teil A* **32**, 171 (1977).
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58. Information on this type of experiment is given by W. T. Silvast, *Sci. Am.* **228**, 88 (February 1973).
59. I thank R. Riley, R. Lang, R. Schmelzer, K. Spielman, T. Weigt, and J. Peters for their assistance in the design and fabrication of our apparatus. I thank P. Brinkman and G. Beenan for their help in preparing this manuscript and J. Schrag, J. Taylor, J. Wright, and D. Evans for helpful comments. This work was supported by the National Science Foundation, the Alfred P. Sloan Foundation, and the University of Wisconsin Graduate School. I particularly thank the Fisher Scientific Company through its Jarrell-Ash Division and F. Brech for the award of a Jarrell-Ash model 750 direct-reading multielement optical spectrometer to this research effort.

H-Y Antigen and the Genetics of Sex Determination

A minimum of three genes may be required for the differentiation of the mammalian testis.

Stephen S. Wachtel

Summary. Widespread phylogenetic conservation of H-Y antigen indicates persistence of a vital function. It has been proposed that this function is the primary determination of mammalian sex. According to this proposal, the indifferent embryonic gonad is induced to differentiate as a testis in the presence of H-Y antigen, and as an ovary in the absence of H-Y antigen. But presence of H-Y antigen does not guarantee testicular differentiation. Other factors may be required: a gene that activates the H-Y structural locus, and another gene that codes for specific H-Y antigen receptors.

Female mice inoculated with cells from males of the same inbred strain produce antibody that identifies a male cell surface component called histocompatibility Y (H-Y) antigen (*1, 2*). Using this antibody in the sperm cytotoxicity test and in another serological assay, the mixed hemadsorption-hybrid antibody test, we have shown that H-Y antigen is widely conserved phylogenetically, occurring in males of all mammalian species so far tested including the human (*3*). On the basis of (i) the evolutionary persistence of H-Y structure (which signifies conservation of a vital function), (ii) the observation that the *Sxr* gene (which reverses the sex of XX mice causing them to develop as males) also specifies H-Y antigen (*4*), and (iii) the putative role of cell-surface components in the cell-cell interactions of organogenesis (*5*), we have proposed that H-Y antigen is the product of the mammalian testis-determining gene (*6*).

According to this proposal, H-Y antigen directs only the initial steps leading to differentiation of the bipotential embryonic gonad as a testis. Further male differentiation is imposed on the embryo by the action of testicular hormones, against the inherent tendency toward the female phenotype (*7*).

Because secondary male sexual differentiation is conferred by the action of testicular hormones, our hypothesis concerning the testis-determining role of H-Y antigen predicts that presence of H-Y should be correlated with presence of at least rudimentary testis regardless of karyotype or phenotypic sex. From this perspective, the genetics of primary (gonadal) sex determination is a simple matter: In the presence of the gene that confers H-Y antigenicity, the indifferent embryonic gonad becomes a testis; in the absence of this gene, the gonad develops as an ovary. But, as the following discussion will show, the genetics of primary sex determination and of H-Y antigen expression is perhaps rather more subtle.

Role of the Y Chromosome in Determination of H-Y Antigen

White blood cells from human males with two Y chromosomes (47,XYY or 48,XXYY) absorb more H-Y antibody than white blood cells from normal 46,XY males (*8*). By implication then, the amount of H-Y antigen on the surface of a cell is directly related to the number of Y chromosomes in the nucleus of that cell. This indicates that a genetic determinant of H-Y antigen expression is on the human Y chromosome, but it does not tell us whether the determinant is a structural gene that specifies the primary structure of H-Y antigen or a regulatory gene that governs the activity of a structural element. The simplest explanation is that the Y-chromosomal H-Y gene is structural, because dosage effect in this case is not easily reconciled with the existence of a Y-situated regulator. At present there is no reason to believe that the products of supernumerary regulatory genes would elicit production of "excess" H-Y antigen, given a single

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structural locus or even multiple structural loci (*9*).

Further evidence that an H-Y gene (whether structural or regulatory) is on the Y chromosome comes from our study of H-Y antigen expression in patients with abnormal Y chromosomes. By correlating presence or absence of H-Y antigen with presence or absence of particular portions of the Y, Koo *et al.* have now obtained data which favor a short arm location near the centromere (*10*). In one case of 17 studied, however, H-Y antigen was present despite the absence of the entire short arm of the Y, an indication that the H-Y locus is on the long arm in at least some individuals (*10*). It is perhaps noteworthy that the two H-Y gene loci (both near the centromere) correspond to two male-determining regions identified by Simpson in an earlier survey (*11*).

H-Y Antigen in XX Males and XX True Hermaphrodites

Testicular differentiation and subsequent male or intersexual development in subjects with female sex chromosomes (XX) is known in several mammalian species. For example the autosomal dominant *Sxr* (sex-reversed) causes both XO and XX mice to develop as phenotypic, albeit sterile, males, this in the absence of any detectable chromosome rearrangement (*12*). As was noted above (*4*), *Sxr* also confers expression of H-Y antigen. It is then necessary to explain expression of H-Y antigen and differentiation of testis in the absence of the Y chromosome. It might be argued that the structural gene for H-Y antigen normally resides on an autosome and that its activating (regulatory) gene is on the Y (*13*). In this context *Sxr* is a constitutive mutant of the autosomal testis-determining H-Y gene although, as I have already stated, this argument is not easily reconciled with the increased dosage of H-Y antigen in males with two Y chromosomes.

Cattanach *et al.*, who first described *Sxr*, suggest that the gene might represent mutational acquisition of male-determining (H-Y determining) function by an autosomal locus or, alternatively, that *Sxr* might represent a Y-to-autosome translocation too small to be detected cytologically (*12*). If it is assumed that *Sxr* originated as a Y-to-autosome translocation, then it must also be assumed that a *chromatid* rather than a chromosome interchange occurred. The point is that *Sxr*/XX males are sterile, and so the *Sxr* gene can only be transmitted by carrier XY males, always along with the Y chromosome that was involved in the original translocation. If the Y chromosome had donated its testis-determining H-Y gene, then it should have lost its testis-determining function. In fact, this Y retains its ability to determine both expression of H-Y antigen and formation of testes.

Ohno has argued that the Y-chromosomal testis-determining gene of mammals exists in multiple copies (*14*). Once the multiple-copy hypothesis is introduced, it is easy to reconcile Y linkage of testis-determining H-Y genes with the Y-to-autosome origin of *Sxr*. In this context, translocation of a critical number of H-Y gene copies would result in both transfer and retention of testis-determining function.

If we assume that there are multiple testis-determining H-Y genes, Y-to-autosome translocation could also explain testicular development in at least some human XX males and XX true hermaphrodites (*15*). In our experience, H-Y antiserum was significantly more cytotoxic after absorption with white blood cells from XX true hermaphrodites than it was after absorption with white blood cells from normal males, indicating reduced expression of H-Y antigen on the cells of these true hermaphrodites (*16*). This could imply Y-to-autosome (or Y-to-X) translocation of some (but not all) H-Y gene copies (*17*). Although the phenomenon of Y-to-autosome translocation remains to be demonstrated karyologically in human XX males or XX true hermaphrodites, cytological evidence has been provided in the study of a 45,X/45,X,22q+ ($H\text{-}Y^+$) male, a case that is perhaps analogous to the situation observed in *Sxr*/XO males (*18*).

Regulation of the H-Y Gene

The Scandanavian wood lemming, *Myopus schisticolor*, is distinguished by an aberrant sex ratio heavily in favor of the female. In this rodent species, many XY embryos develop as anatomically normal, fertile females (*19*); ovarian differentiation and subsequent feminization proceed in the presence of an intact Y chromosome but in the absence of H-Y antigen (*20*). The XY female wood lemming condition cannot be due to mutation or loss of relevant Y-chromosomal genes, however, because the exceptional females, though somatically XY, have an XX germ line. They cannot transmit their Y. In fact, the XY female condition is transmitted as an X-linked trait, and this indicates existence of an X-chromosomal gene that can suppress or modify the activity of testis-determining (H-Y) structural genes (*20*).

In view of the conservatism of the mammalian X chromosome (*14*), a similar X-chromosomal gene may occur in other mammalian species, including the human (*21*). Whereas XO rodents are fertile females, XO humans are sterile females with streak gonads. Thus, we can predict the familial occurrence of a human X-linked condition in which affected individuals, 46,XY, develop as $H\text{-}Y^-$ phenotypic females with streak gonads (pure gonadal dysgenesis) (*22*). Sporadic cases of pure gonadal dysgenesis could arise as a consequence of spontaneous mutations of the hypothetical X-linked regulatory locus. If it is assumed that a single H-Y structural locus exists, then mutation at this locus could also account for sporadic cases of pure gonadal dysgenesis. If, in contrast, the Y-linked H-Y structural locus occurs in multiple copies, then loss of a sufficient portion of these copies should also affect expression of H-Y antigen and, by inference, differentiation of the testis. Indeed, one of the products of any Y-to-autosome or Y-to-X translocation is a defective Y chromosome, and XY gonadal dysgenesis with some virilization of the streak gonads in the presence of "intermediate" levels of H-Y antigen might be explained in terms of such an event.

But presence of H-Y antigen alone need not signify simultaneous presence of virilized gonads. Undifferentiated streak gonads have been found in several phenotypic females whose white blood cells expressed H-Y antigen. In one of these subjects, a structure resembling an epididymis was observed in the left gonadal ridge, indicating that testicular tissue may have been present at one time, and karyotypic analysis of cultured cells from one of the streak gonads of another $H\text{-}Y^+$ phenotypic female revealed what had been a "cryptic" 45,XO cell line only, an indication that, in this case, lack of testicular differentiation was due to presence of the aneuploid line in the presumptive gonad during the relevant stages of embryogenesis.

Other Genes

Still, undefined "regression" of testicular structures and mosaicism may not account for all cases of pure gonadal dysgenesis in man. An example is the $H\text{-}Y^+$ female who develops streak gonads and no internal male structures in the absence of any detectable sex-chromosome

mosaicism. While it is difficult to rule out the specter of cryptic mosaicism, it is also difficult to envision any morphogenetic function of H-Y antigen that is independent of a specific plasma membrane receptor (*23*). And given a receptor, it is necessary to postulate a gene that codes for its structure. But once the concept of a gene that codes for an H-Y antigen-receptor is introduced, we are confronted with the possibility (or perhaps the inevitability) of mutation of this gene. Let us assume that such a mutation occurs in an otherwise "normal" 46,XY embryo. H-Y antigen would be present, but testicular differentiation could not proceed normally, and the affected individual ($H\text{-}Y^+$) would be expected to develop streak gonads exhibiting some or no virilization, depending on any residual ability of the mutated receptor to bind H-Y antigen (or on any residual ability of the gonad itself to virilize in the absence of an "ovarian inducer") (*24*).

Conclusion

Although H-Y antigen is not of itself sufficient to guarantee masculinization of the indifferent gonad, its presence seems necessary in order for normal testicular differentiation to occur. Thus, genetic or environmental factors that affect the expression and action of H-Y antigen probably affect normal development of the gonad as well. In the context of our foregoing discussion, some of these factors can be classified as follows: (i) mutation or loss of H-Y structural determinants, (ii) position effects resulting from translocation of H-Y structural genes, (iii) mutation of H-Y activating genes, and (iv) mutation of genes that specify plasma membrane receptors for H-Y antigen. It is perhaps appropriate to mention that just as expression of H-Y antigen does not always signal testicular differentiation, absence of H-Y does not always signal normal ovarian differentiation (which is dependent, so far as we know, on the presence of two X chromosomes in man).

The ultimate value of H-Y antigen serology as a diagnostic measure remains to be determined. Certainly H-Y antigen expression implies presence of Y-chromosomal genes and simultaneous presence of streak gonads, or testes, but its expression in blood cannot preclude the presence of ovaries in a female or intersexual patient with XX/XY sex chromosomal mosaicism, for example. It is sufficient to say that at present H-Y antigen serology can serve as a valuable adjunct to more routine karyologic and endocrinologic measures, and that, in conjunction with these, study of H-Y antigen may ultimately broaden our understanding of both normal and abnormal sexual differentiation not only in man, but in mammals generally.

References and Notes

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6. S. Ohno, *ibid.* **7**, 315 (1976); S. S. Wachtel, S. Ohno, G. C. Koo, E. A. Boyse, *Nature (London)* **257**, 235 (1975); also see (*1*).
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9. This assumes that what we recognize serologically as H-Y antigen is a single molecule (? polypeptide) coded by genetically homogeneous structural loci, and that it is not a collection of macromolecular subunits, each exhibiting a discrete antigenic specificity, and each coded by a discrete structural locus.
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13. The possibility that the gene is associated physically with H-2 is discussed by Silvers and Wachtel (*1*). But linkage between *Sxr* and H-2 has been excluded [B. M. Cattanach, in *The Early Development of Mammals*, M. Balls and A. Wild, Eds. (Cambridge Univ. Press, Cambridge, 1975), p. 305].
14. S. Ohno, *Sex Chromosomes and Sex-Linked Genes* (Springer-Verlag, New York, 1967).
15. Y-to-autosome translocation cannot account for all XX males and XX true hermaphrodites in man; sex-chromosome mosaicism (one cell line having a Y chromosome, another lacking it) and Y-to-X interchange have both been implicated in separate studies [see discussion in (*16*)].
16. S. S. Wachtel, G. C. Koo, W. R. Breg, H. T. Thaler, G. M. Dillard, I. M. Rosenthal, H. Dosik, P. S. Gerald, P. Saenger, M. New, E. Lieber, O. J. Miller, *N. Engl. J. Med.* **295**, 750 (1976).
17. The true hermaphrodite is a mosaic; some cells organize testis while others organize ovary often within the same gonad. Reduced expression of H-Y antigen in the white blood cells of XX true hermaphrodites might therefore reflect $H\text{-}Y^+/H\text{-}Y^-$ mosaicism, some cells possessing H-Y structural determinants, others lacking them. But it could also reflect a decrease in the amount of H-Y antigen per cell because of the absence of some H-Y structural gene copies.
18. In this case, a minute piece of additional material was translocated to one chromosome 22 in some cells. The size of the additional band approximated the size of the short arm of the Y. An intact Y chromosome was not found in any cell line [G. C. Koo, S. S. Wachtel, W. R. Breg. O. J. Miller, *Birth Defects Orig. Art. Ser.* **12**, 175 (1976)].
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25. Supported in part by NIH grants CA 08748, AI 11982, HD 00171, and HD 10065.