Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 3368–3372, April 1993
Genetics

A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development

(hermaphroditism/Y chromosome/sex reversal)

KEN MCELREAVEY*, ERIC VILAIN*, NACER ABBAS*, IRA HERSKOWITZ†, AND MARC FELLOUS*‡

*Laboratoire d'Immunogenetique Humaine, Institut National de la Santé et de la Recherche Médicale U.276, Institut Pasteur, 25/28 rue du Dr. Roux, 75724 Paris Cedex 15, France; and †Department of Biochemistry and Biophysics, School of Medicine, University of California, San Francisco, San Francisco, CA 94143-0448

Contributed by Ira Herskowitz, January 4, 1993

ABSTRACT **The mammalian Y chromosome carries the *SRY* gene, which determines testis formation. Here we review data on individuals who are XX but exhibit male characteristics: some have *SRY*; others do not. We have analyzed three families containing more than one such individual and show that these individuals lack *SRY*. Pedigree analysis leads to the hypothesis that they carry recessive mutations (in a gene termed *Z*) that allow expression of male characteristics. We propose that wild-type Z product is a negative regulator of male sex determination and is functional in wild-type females. In males, SRY product represses or otherwise negatively regulates Z and thereby allows male sex determination. This hypothesis can also explain other types of sex reversal in mammals, in particular, XY females containing *SRY*. Some of these individuals may have mutations at the *Z* locus rendering them insensitive to SRY. Recessive mutations (such as the *polled* mutation of goats) leading to sex reversal are known in a variety of animals and might be used to map and ultimately clone the human *Z* gene.**

In mammals, male sex determination is controlled by the presence of the Y chromosome, which carries a gene encoding the testis-determining factor (*TDF*) on its short arm (1, 2). The current hypothesis for *TDF* action is that its product induces the testis-determination pathway, resulting ultimately in a male phenotype (1, 3–6). Recently, the testis-determining factor gene has been isolated and named *SRY* for sex-determining region, Y chromosome (7). Genetic evidence to equate *SRY* and *TDF* comes from the study of XY sex-reversed females who harbor *de novo* mutations in the *SRY* open reading frame (ORF). The association of a *de novo* mutation in the *SRY* ORF with a sex-reversed phenotype is consistent with *SRY* being *TDF* (8–12). Further support for the role of *SRY* as the primary testis-determining factor comes from the observation that XX mice carrying the murine equivalent of *SRY* as a transgene develop as males (13).

Mutations that cause sex reversal have proved to be important for identifying genes involved in sex determination in *Drosophila*, nematodes, and mice (5, 14). We have therefore sought to examine cases of sex reversal in humans that are not explained simply by alterations in *SRY* (mutations in the *SRY* gene that alter phenotypes of XY individuals or translocations of *SRY* that alter phenotypes of XX individuals). Here we provide a critical reevaluation of >100 cases of XX individuals who express male phenotypes, either giving a full male phenotype (male without ambiguities) or a less complete male phenotype (male with ambiguities or 46,XX true hermaphrodite). These cases, both sporadic and familial, do not show a simple relationship between the presence of *SRY* and male gonad phenotype. In particular, pedigree analysis reveals families that lack *SRY* but that exhibit XX sex reversal. These pedigrees indicate that male sex determination can occur in the absence of *SRY* if individuals are defective in a gene termed *Z*. We propose that SRY protein activates male sex determination by blocking synthesis or activity of Z protein, which is a negative regulator of male sex determination. Because SRY protein contains a DNA-binding domain (7, 15–17), it may function as a repressor of synthesis of Z.

MATERIALS AND METHODS

Patients. *XX males without ambiguities.* Thirty-five cases of XX males (18) have been clinically, biologically, and cytogenetically analyzed using Y-specific DNA probes. A number of these have been described (19, 20). All 35 cases were phenotypically male without external or internal genital ambiguities. The karyotype in each case was 46,XX without detectable mosaicism.

XX males with genital ambiguities: Sporadic and familial cases. Thirty-eight sporadic cases of XX males were analyzed for the presence of Y material (21). They were detected at birth because of external genital ambiguities, such as hypospadias (failure of urethra to close on the underside of the penis), micropenis, or hyperclitoridy. In all 38 cases, gonadal biopsy revealed the presence of bilateral testis. We also analyzed a familial case (family A) in which two children were XX males with ambiguities such as posterior hypospadias. As shown in Fig. 1, they have two normal brothers and five normal sisters. Both parents are normal; they are related by a first-cousin marriage.

46,XX true hermaphrodites: Sporadic and familial cases. Thirty-four sporadic cases have been analyzed; the majority have been previously published (21, 22). They all carried internal or external genital ambiguities detected at birth. The differential diagnosis with respect to the other classes of XX males was made by gonadal biopsy, which demonstrated the presence of testicular and ovarian tissue. We have also studied two familial cases. Fig. 2 shows family P, with two affected children—both 46,XX true hermaphrodites with a bilateral ovotestis. Fig. 3 shows family J, with two affected children—one a 46,XX true hermaphrodite with a bilateral ovotestis and the other an XX male with ambiguities but with a bilateral testis. No consanguinity was found in family J or family P.

DNA Analysis. All 107 cases of XX sex reversal were analyzed by Southern blotting using probes specific for the Y chromosome (HF0.2, pY53.3, PO.9, DYS104, DYS13, and ZFY) and covering the long and short arms, including *SRY*, *ZFY*, and the pseudoautosomal Y boundary locus (7, 23–26). The genetic organization of the short arm of the Y chromosome is telomere–HF0.2–pY53.3(*SRY*)–PO.9–DYS104–

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed.

Table 1. Presence or absence of *SRY* in patients with XX male pathology

Patient	*SRY*	
	Present	Absent
XX male without ambiguities	32 (28 also contain *ZFY*)	3
XX male with ambiguities	2 (1 also contains *ZFY*)	36
46,XX true hermaphrodite	4 (1 also contains *ZFY*)	30

Data for sporadic and familial cases are pooled. Additional information on these patients is provided in the text.

DYS13–ZFY–centromere. Probes used for the long arm of the Y chromosome were DYS1 and DYS11 (27).

The primers for specific amplification of the Y pseudoautosomal boundary were as follows: A, GTACTACCTTTAGAAAACTAGTATTTTCCC, and C, GAATTCTTAACAGGACCCATTTAGGATTAA. Amplification was carried out as described by Ellis *et al.* (26). The primers used for *SRY* amplification were as follows: SRYEA, GGAATTCCCTAACTCTAAGTATCAGTGT, and SRYEB, GGAATTCCGCAAACTGCAATTCTTCGGC. Amplification was carried out as described by Vilain *et al.* (28).

RESULTS

Analysis of Sporadic Cases of 46,XX Sex-Reversed Individuals for Presence of *SRY*. Sex-reversed XX individuals can be categorized into three types depending on their clinical phenotypes (see *Materials and Methods*): XX males without ambiguities of the external or internal genitalia, XX males with ambiguities, and 46,XX true hermaphrodites. The presence of *SRY* in these three types is shown in Table 1. Approximately 90% of XX males without ambiguities carried *SRY*; 28 of these 32 also carried another Y-specific marker, *ZFY*. Three of the XX males without ambiguities lacked all Y-specific sequences tested, including *SRY*. In two of these cases, we had access to gonad biopsy material and could exclude Y chromosome mosaicism by PCR analysis using Y-specific primers (see *Materials and Methods*).

In contrast, the majority of the other two classes of sex-reversed individuals lacked *SRY*: 36 of 38 XX males with ambiguities lacked *SRY*; of the two that carried *SRY*, one also carried *ZFY*. Thirty of 34 46,XX true hermaphrodites lacked *SRY*; of the four that carried *SRY*, one also contained *ZFY*.

In general, more complete sex reversal was seen in individuals who carried *SRY* than in those who lacked *SRY*: 32 of 38 carrying *SRY* exhibited a male phenotype without ambiguities, whereas only 3 of 69 lacking *SRY* exhibited this phenotype. The exceptions to the general rule are discussed below.

Analysis of Familial Cases of Sex-Reversed Females for Presence of *SRY*. Three families have been identified that contain more than one sex-reversed individual having the XX male with ambiguities or 46,XX true hermaphrodite phenotypes. For example, in family A, two of the nine children are XX males with ambiguities (Fig. 1). Southern hybridization using the probe pY53.3, which contains *SRY*, showed that these sex-reversed individuals lacked *SRY* (Fig. 2, lanes 4 and 5). A normal *SRY*-containing band was observed in the father (Fig. 2, lane 1). Similar results were obtained for another family (family P; Fig. 3) with two 46,XX true hermaphrodite children: *SRY* is absent (Fig. 2, lanes 8 and 9). Family J (Fig. 4) contains one child who is an XX male with ambiguities and a sibling who is a 46,XX true hermaphrodite, both of whom lack *SRY* (data not shown).

DISCUSSION

Pedigree Analysis Indicates the Existence of a Gene, *Z*, that Negatively Regulates Male Sexual Development—a Regulatory Cascade for Sex Determination. A survey of XX sex reversal reveals many cases in which testicular tissue is present in the absence of Y DNA material, in particular, in the absence of *SRY*. Perhaps most striking is the identification of three families in which XX sex reversal is observed in two children. Because the parents and other ancestors did not exhibit sex-reversed phenotypes, it is simplest to interpret these pedigrees in terms of a single autosomal locus, *Z*, whose recessive allele confers male phenotypes even in the absence of *SRY*. Thus, the parents in family A (Fig. 1) are both proposed to be Z^+/Z^- heterozygotes, and the affected children to be XX Z^-/Z^- homozygotes.

More complex genetic hypotheses are possible. For example, it is formally possible that the mutation responsible for sex reversal is a dominant mutation carried by the father on an autosome or on the pseudoautosomal region of the Y chromosome (so that it can be passed to XX children). One would have to argue that this dominant mutation is newly arisen in the father or has escaped notice in earlier generations because it is present only in XY individuals, where it has no phenotype. We consider this explanation unlikely and instead favor the view that this sex reversal results from simple loss-of-function mutations at the *Z* locus. The proposal that recessive mutations can be responsible for XX sex reversal in humans has been suggested (29–31). XX sex reversal has also been described in domestic animals such as pig, goat, and dog, where in some cases, it has been attributed to a monofactorial recessive mode of inheritance (32–36).

Classical genetic studies of mammalian sex determination lead to the view that the testis-determining gene carried on the Y chromosome is necessary for activating a cascade of genes involved in male gonad formation (5, 6). The phenotype of human mutants defective in the *SRY* gene and the behavior of transgenic mice containing the mouse *Sry* gene (7, 13) indicate that the *SRY* gene encodes the testis-determining factor and that its presence triggers male sex determination. The SRY protein is thus formally a positive regulator of male

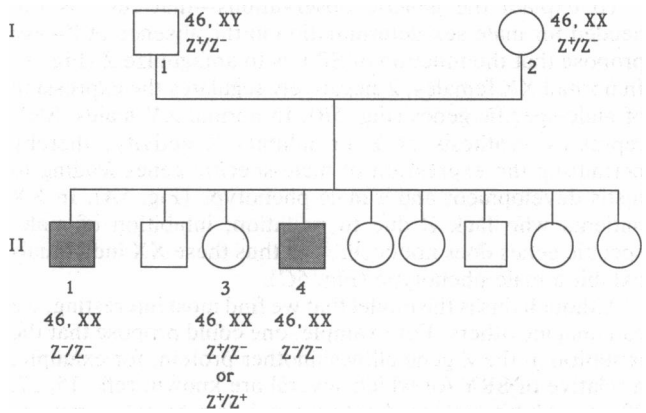

FIG. 1. Family A, with two cases of XX males with genital ambiguities. Open circles, normal females; open squares, normal males; filled squares, XX males with ambiguities (posterior hypospadias). DNA from parents (I-1 and I-2), the two patients (II-1 and II-4), and unaffected sister (II-3) is analyzed in Fig. 2 for the presence of *SRY*. Z^+ and Z^- are inferred genotypes.

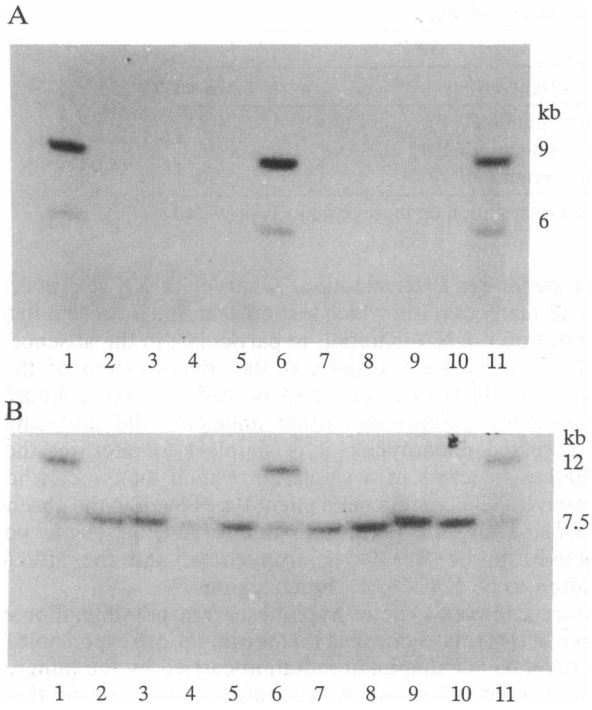

FIG. 2. Southern blot analysis of patients exhibiting XX male pathology for presence of the *SRY* gene. DNA samples were obtained from the individuals indicated below and probed an *SRY*-containing DNA probe (*A*) and a *ZFY*-containing probe as a positive control (*B*). Probes are described in the text. Family A: father (I-1, lanes 1), mother (I-2, lanes 2), unaffected sister (II-3, lanes 3), XX male with ambiguities (II-1, lanes 4), XX male with ambiguities (II-4, lanes 5). Family P: father (II-1, lanes 6), mother (II-2, lanes 7), XX male with ambiguities (III-2, lanes 8), XX male with ambiguities (III-3, lanes 9). Controls: normal female (lanes 10), normal male (lanes 11). The results of *SRY* analysis for family P have been described by McElreavey *et al.* (22). The *ZFY*-containing probe also recognizes the X homologue *ZFX*. Hence, all individuals have a fragment of 7.5 kb, and XY individuals have a fragment of 12.5 kb. The fragments of 2.3 kb and 1.9 kb also recognized by the *ZFY*-containing probe are not shown.

sex determination. But how does it functional mechanistically? The nucleotide sequence shows that it contains a sequence like that in a family of DNA-binding proteins (7). Indeed, SRY has now been shown to be capable of binding DNA (15–17).

To explain the genetic observations—that SRY is not needed for male sex determination in the absence of Z—we propose that the function of SRY is to antagonize Z (Fig. 5). In normal XX females, Z negatively regulates the expression of male-specific genes (Fig. 5*B*). In normal XY males, SRY represses synthesis of Z or inhibits Z activity, thereby permitting the expression of male-specific genes leading to testis development and a male phenotype (Fig. 5*A*). In XX patients who lack *Z* due to mutation, inhibition of male-specific genes does not occur, and thus these XX individuals exhibit a male phenotype (Fig. 5*C*).

Although this is the model that we find most interesting, we can imagine others. For example, one could propose that the mutation in the *Z* gene allows another protein, for example, a relative of SRY (of which several are known; refs. 15, 37, 38), to exhibit increased activity and thereby take over the functional role of SRY to some extent.

Genetic Predictions of the Hypothesis. We interpret the sex-reversed phenotype observed in families A, J, and P to have resulted from recessive mutations in the *Z* gene. We do not know the nature of the mutations in sporadic cases of XX sex reversal: they may also be recessive, but there may be

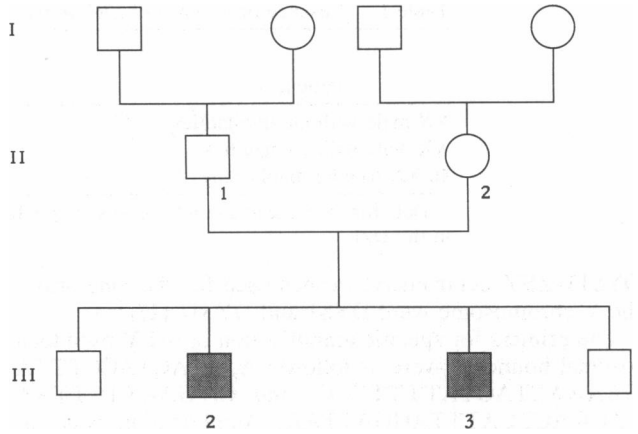

FIG. 3. Family P, with two cases of 46,XX true hermaphrodites. Symbols are as described in the legend to Fig. 1. Analysis of the two affected children and their parents for *SRY* is shown in Fig. 2.

dominant negative mutations as well. Skordis *et al.* (39) have previously reported a family containing XX males and 46,XX true hermaphrodites occurring in two generations of the family that they interpreted as due to an autosomal dominant mode of inheritance. These individuals have been shown to lack *SRY* (M. MacGillivray and H. Ostrer, personal communication); they may carry a dominant negative allele of *Z*.

The cascade hypothesis allows the prediction of the consequences of a mutation in *Z*, which we can call Z^i, such that it is insensitive to SRY action. Because SRY is a DNA-binding protein, it might function by inhibiting synthesis of Z (other possibilities are discussed below). Z^i mutations might thus be insensitive to this inhibition—in other words, operator constitutive mutations (40). These mutations would have very different properties from the Z^- mutations: Z^i mutations would be dominant and result in XY individuals exhibiting female characteristics (Fig. 5*D*). In recent studies, we have found that 80% of XY females with pure gonadal dysgenesis syndrome have a wild-type *SRY* gene (11, 12). Some of these individuals might have a Z^i mutation.

Eicher and Washburn (5) have described autosomal loci in the mouse that result in sex reversal of XY animals: they exhibit inappropriate female phenotypes. In particular, they have identified a locus, *Tda-1*, that determines the ability of the testis-determining factor of the Y chromosome from one genetic background to function in another genetic background. Could the *Tda-1* locus code for a Z^i allele—a *Z* locus that is refractory to inhibition by some mouse Sry proteins

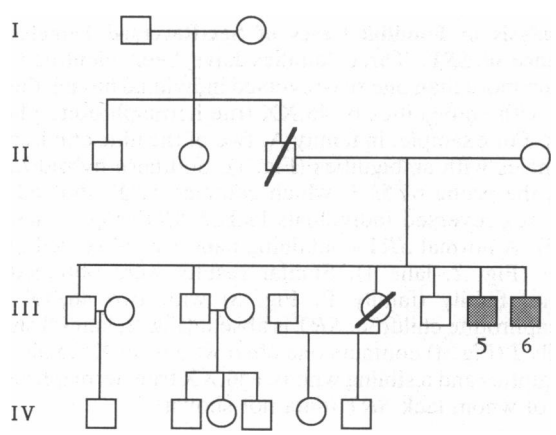

FIG. 4. Family J, with one case of XX male with ambiguities and one case of 46,XX true hermaphrodite. Symbols are as described in the legend to Fig. 1. Patient III-5 is a 46,XX true hermaphrodite. Patient III-6 is an XX male with ambiguities.

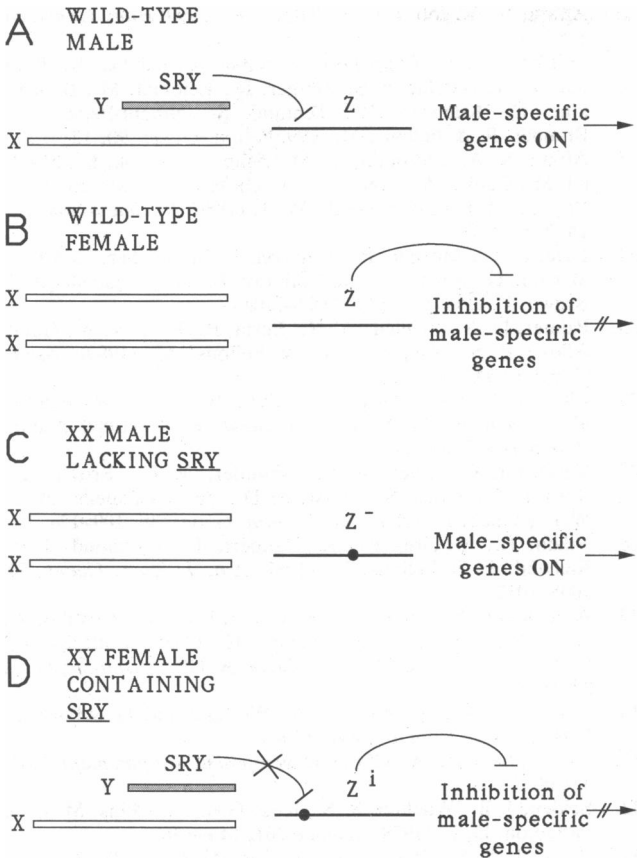

FIG. 5. Regulatory cascade hypothesis for mammalian sex determination and an explanation for sex reversal in XX *SRY*$^-$ and XY *SRY*$^+$ individuals. (*A*) In wild-type males, SRY protein is present and inhibits Z by acting as a repressor of Z synthesis (as drawn) or by inhibiting activity of Z (not drawn). Male sex determination is therefore uninhibited by Z and can proceed. (*B*) In wild-type females, SRY protein is lacking. Therefore, Z protein is able to inhibit male sex determination, and female sex determination occurs. (*C*) XX individuals lacking *SRY* who exhibit male phenotypes (male with sexual ambiguities or true hermaphroditism) are proposed to be defective in the *Z* gene due to being homozygous for a Z^- mutation (indicated by ●). These individuals are therefore able to express male sex determination. (*D*) XY individuals who carry an intact *SRY* gene can exhibit female phenotypes if they carry a mutation at the *Z* locus (Z^i, indicated by ●) that renders *Z* insensitive to repression by SRY protein. Z protein is therefore produced and can inhibit male sex determination, allowing female sex determination to occur.

but not by others? This explanation appears unlikely because the *Tda-1*^{b} allele is recessive whereas Z^i is expected to be dominant. Although explanations for the interaction of *Tda-1* and *Sry* have been proposed (5, 41), we can suggest others in the context of the regulatory cascade hypothesis presented here. For example, Tda-1 protein might be necessary for transcription of the *Sry* gene or it might be a corepressor that works in conjunction with Sry to inhibit Z synthesis or activity. Some *SRY*$^+$ XY humans who exhibit sex reversal might carry loss-of-function mutations in a human *Tda-1* homologue.

Mechanistic and Functional Implications of the Regulatory Cascade Hypothesis. We presented above the view that SRY protein might be a repressor that controls synthesis of Z protein, which is a negative regulator of male-specific sex determination. Another possibility is that SRY controls activity rather than synthesis of Z protein. For example, Z might inhibit transcription of male-specific genes, and SRY might compete with Z for its binding site. Cloning of the *Z* gene would, of course, help to distinguish between these hypotheses. If SRY represses transcription of *Z*, then the *Z* gene might be cloned as a female-specific cDNA.

Whether SRY acts on one or more than one target is an important question. In the presence of *SRY*, sex reversal of XX individuals is generally complete, and a phenotype of male without sexual ambiguities is typically seen. (The rare cases of incomplete sex reversal despite the presence of *SRY* might result from reduced levels of *SRY* expression in these presumed translocations, analogous to what is argued for some transgenic XX mice carrying *SRY*, which do not exhibit male phenotypes; ref. 13.) In contrast, the range of phenotypes in XX sex-reversed individuals who lack SRY is considerable: from a male phenotype with some ambiguities to a true hermaphrodite who can be phenotypically female. We can imagine two types of explanation for the incomplete sex reversal: SRY might have more than one target, for example, *Z* and *A*, which code for independent negative regulators of male development. Even if this were the case, the considerable extent of male phenotypes observed in XX Z^- individuals would indicate that a major action of SRY is to inhibit Z. The great variability of phenotypes seen in these individuals can be easily explained by proposing that the different Z^- mutations are leaky to different extents, in other words, that they have some residual activity.

Although, in general, XX individuals lacking *SRY* exhibit a less complete sex reversal than XX *SRY*$^+$ individuals, three cases of XX males without ambiguities have been noted in XX *SRY*$^-$ individuals. These rare individuals may carry a null mutation in the *Z* gene, whereas the XX Z^- individuals who exhibit less complete sex reversal may have leaky *Z* mutations. Of course, we cannot exclude the possibility that the complete sex reversal of the minority XX *SRY*$^-$ individuals results from other mutations in the male-determination pathway or from a mutation in an *SRY*-related gene that allows it to mimic SRY action.

The regulatory cascade hypothesis may be tested using two distinct approaches. First, cloning of target genes regulated by SRY might identify the *Z* gene itself. Second, a candidate *Z* gene might be mapped using the pig intersexuality mutation as an animal model for human XX sex reversal. Genetic analysis of intersexuality in the pig is compatible with an autosomal recessive mutation (34). Since the pig map is becoming well defined (42), this mutation can be mapped using Mendelian genetics and its equivalent can be located in humans or in mice using a comparative mapping approach. If we are correct that some cases of human XY females are due to mutations at the *Z* locus (the Z^i mutations), then mapping information for these types of mutations could be pooled with data for the Z^- mutations that cause XX sex reversal; this might allow mapping of the *Z* locus directly in humans.

Analogies with Other Gene Control and Sex-Determination Systems. Our proposal that a positive regulator, SRY, might function mechanistically by inhibiting an inhibitor has precedent from other organisms. For example, the yeast regulatory protein a1-α2 triggers initiation of meiosis by repressing transcription of an inhibitor of meiosis (the RME1 product; ref. 43; see also ref. 44). The sex-determination pathways of *Drosophila* and nematodes begin by monitoring chromosomal ratios, which then leads to a multistep pathway governing activity of distal regulatory proteins. Many of the steps in the pathway involve inhibitors of inhibitors and are thus analogous to the proposal for the first steps of mammalian sex determination. As noted by Hodgkin (45), these analogies do not hold up to detailed molecular scrutiny and may reflect independent evolution of similar strategies rather than exploitation of the same molecular machinery.

In conclusion, the two-gene hypothesis described here satisfactorily explains all known pathologies of human sex determination. Testing of this hypothesis will require cloning of the *Z* gene, which might be a direct target of SRY.

We thank Drs. C. Boucekkine, C. Moreira-Filho, K. Kucheria, R. Brauner, R. Rappaport, and J. E. Toublanc for their support of this work and F. Jacob for comments on the manuscript. We also thank Nicole Souleyreau for excellent technical assistance and we thank M. MacGillivray and H. Ostrer for their unpublished information. This work was supported by Institut National de la Santé et de la Recherche Medicale reseau Nord/Sud and Fondation pour la Recherche Medicale. I.H. thanks Professor F. Jacob and the Collège de France for support during a stay in Paris.

1. Burgoyne, P. S. (1988) *Philos. Trans. R. Soc. London B* **322,** 63–72.
2. McLaren, A. & Ferguson-Smith, M. A. (1988) *Philos. Trans. R. Soc. London B* **322,** 1–157.
3. Jost, A. (1947) *Arch. Anat. Microsc. Morphol. Exp.* **36,** 271–315.
4. Goodfellow, P. N. & Darling, S. M. (1988) *Development* **102,** 251–258.
5. Eicher, E. M. & Washburn, L. L. (1986) *Annu. Rev. Genet.* **20,** 327–360.
6. McLaren, A. (1991) *Oxford Rev. Reprod. Biol.* **13,** 1–33.
7. Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., Foster, J. W., Frischauf, A.-M., Lovell-Badge, R. & Goodfellow, P. N. (1990) *Nature (London)* **346,** 240–244.
8. Berta, P., Hawkins, J. R., Sinclair, A. H., Taylor, A., Griffiths, B. L., Goodfellow, P. N. & Fellous, M. (1990) *Nature (London)* **348,** 448–450.
9. Jäger, R. J., Anvret, M., Hall, K. & Scherer, G. (1990) *Nature (London)* **348,** 452–454.
10. Hawkins, J. R., Taylor, A., Berta, P., Levilliers, J., Van der Auwera, B. & Goodfellow, P. N. (1992) *Hum. Genet.* **88,** 471–474.
11. McElreavey, K., Vilain, E., Boucekkine, C., Vidaud, M., Jaubert, F., Richaud, F. & Fellous, M. (1992a) *Genomics* **13,** 838–840.
12. McElreavey, K., Vilain, E., Abbas, N., Costa, J.-M., Souleyreau, N., Kucheria, K., Boucekkine, C., Thibaud, E., Brauner, R., Flamant, F. & Fellous, M. (1992b) *Proc. Natl. Acad. Sci. USA* **89,** 11016–11020.
13. Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. & Lovell-Badge, R. (1991) *Nature (London)* **351,** 117–121.
14. Hodgkin, J. (1990) *Nature (London)* **344,** 721–728.
15. Nasrin, N., Buggs, C., Kong, X. F., Carnazza, J., Goebl, M. & Alexander-Bridges, M. (1991) *Nature (London)* **354,** 317–320.
16. Harley, V. R., Jackson, D. I., Hextall, P. J., Hawkins, J. R., Berkovitz, G. D., Sockanathan, S., Lovell-Badge, R. & Goodfellow, P. N. (1992) *Science* **255,** 453–456.
17. Giese, K., Cox, J. & Grosschedl, R. (1992) *Cell* **69,** 185–195.
18. de la Chapelle, A. (1981) *Hum. Genet.* **58,** 105–116.
19. Seboun, E., Leroy, P., Casanova, M., Magenis, E., Boucekkine, C., Disteche, C., Bishop, C. & Fellous, M. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51,** 237–248.
20. Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M. & Weissenbach, J. (1984) *Nature (London)* **307,** 172–173.
21. Abbas, N. E., Toublanc, J. E., Boucekkine, C., Toublanc, M., Affara, N. A., Job, J. C. & Fellous, M. (1990) *Hum. Genet.* **84,** 356–360.
22. McElreavey, K., Rappaport, R., Vilain, E., Abbas, N., Richaud, F., Lortat-Jacob, S., Berger, R., Leioniat, M., Boucekkine, C., Kucheria, K., Tentami, S., Nihoul-Fekete, C., Brauner, R. & Fellous, M. (1992) *Hum. Genet.* **90,** 121–125.
23. Affara, N. A., Florentin, L., Morrison, N., Kwok, K., Mitchell, M., Cooke, A., Jamieson, D., Glasgow, L., Meredith, L., Boyd, E. & Ferguson-Smith, M. A. (1986) *Nucleic Acids Res.* **14,** 5353–5373.
24. Page, D. C., Mosher, R., Simpson, E. M., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A. & Brown, L. (1987) *Cell* **51,** 1091–1104.
25. Palmer, M. S., Sinclair, A. H., Berta, P., Ellis, N. A., Goodfellow, P. N., Abbas, N. E. & Fellous, M. (1989) *Nature (London)* **342,** 937–939.
26. Ellis, N. A., Goodfellow, P. J., Pym, B., Smith, M., Palmer, M., Frischauf, A.-M. & Goodfellow, P. N. (1989) *Nature (London)* **337,** 81–84.
27. Vergnaud, G., Page, D. C., Simmler, M. C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A. & Weissenbach, J. (1986) *Am. J. Hum. Genet.* **38,** 109–124.
28. Vilain, E., McElreavey, K., Jaubert, F., Raymond, J.-P., Richaud, F. & Fellous, M. (1992) *Am. J. Hum. Genet.* **50,** 1008–1011.
29. Armendares, S., Salamanca, F., Cantu, J. M., Del Castillo, V., Nava, S., Dominguez-de-la-Piedra, E., Cortes-Gallegos, V., Gallegos, A., Cervantes, C. & Parra, A. (1975) *Humangenetik* **29,** 99–109.
30. de la Chapelle, A., Tippett, P. A., Wetterstrand, G. & Page, D. (1984) *Nature (London)* **307,** 170–171.
31. de la Chapelle, A. (1987) *Development (Supplement)* **101,** 33–38.
32. Selden, J. R., Wachtel, S. S., Koo, G. C., Haskins, M. E. & Patterson, D. F. (1978) *Science* **201,** 644–646.
33. Wachtel, S. S., Basrur, P. & Koo, G. C. (1978) *Cell* **15,** 279–281.
34. Sittmann, K., Breeuwsma, A. J. & te Brake, J. H. A. (1980) *Can. J. Genet. Cytol.* **22,** 507–527.
35. Cribiu, E. P. & Chaffaux, S. (1990) *Reprod. Nutr. Dev. Suppl.* **1,** 51s–61s.
36. Hamerton, J. L., Dickson, J. M., Pollard, C. E., Grieves, S. A. & Short, R. V. (1969) *J. Reprod. Fert. Suppl.* **7,** 25–51.
37. Travis, A., Amsterdam, A., Belanger, C. & Grosschedl, R. (1991) *Genes Dev.* **5,** 880–894.
38. Castrop, J., van Norren, K. & Clevers, H. (1992) *Nucleic Acids Res.* **20,** 611.
39. Skordis, N. A., Stetka, D. G., MacGillivray, M. H. & Greenfield, S. P. (1987) *J. Pediatr.* **110,** 244–248.
40. Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3,** 318–356.
41. Washburn, L. L. & Eicher, E. M. (1989) *Genetics* **123,** 173–179.
42. O'Brien, S. J. & Graves, J. A. (1991) *Cytogenet. Cell. Genet.* **58,** 1124–1151.
43. Mitchell, A. P. & Herskowitz, I. (1986) *Nature (London)* **319,** 738–742.
44. Banuett, F. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3922–3926.
45. Hodgkin, J. (1990) *Nature (London)* **344,** 721–728.