Journal of Steroid Biochemistry, 1977, Vol. 8, pp 585–592. Pergamon Press. Printed in Great Britain

THE Y-LINKED H-Y ANTIGEN LOCUS AND THE X-LINKED *Tfm* LOCUS AS MAJOR REGULATORY GENES OF THE MAMMALIAN SEX DETERMINING MECHANISM

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SUMMARY

The mammalian embryo has an inherent tendency to develop as a female. The male development is due to interventions by two major regulatory genes. Thus, these two genes should be regarded as the primary regulatory loci of the mammalian sex determining mechanism. The first gene is Y-linked and apparently specifies a plasma membrane protein known as H-Y antigen. The role of this gene is to induce testicular organization by an embryonic indifferent gonad.

The Y-induced testis then produces androgen and all extra-gonadal masculine developments are induced by androgen. The responsiveness of all types of target cells to androgen with a possible exception of male germ cells is mediated by the X-linked *Tfm* locus which is believed to specify the nuclear-cytosol andogen-receptor protein.

In mammals, 2AXO (the diploid set of autosomes plus a single X) represents the minimal viable genetic constitution and this constitution gives rise not to an asexual individual but to a female. Hence, an oft repeated statement that the embryonic plan of mammals is inherently feminine. The male development is caused by successive interventions of this basic plan by two major regulatory elements. First, a gene or genes that reside on the mammalian Y-chromosome divert an embryonic indifferent gonad's natural inclination toward ovarian-development, and direct it to form a testis. This apparently is all that the Y-linked first regulatory element does. Witness the fact that in spite of the normal testicular organization by the Y, no further masculine development occurs in XY individuals that carry testicular feminization mutation.

The link between the first regulatory element which is Y-linked and the second regulatory system which is under control of the X-linked gene is provided by the fact that the testis produces an inducer of this second regulatory system. Although the mammalian fetal body has an inherent tendency to develop along the feminine direction, the XX or XY body readily masculinizes when exposed to testosterone. The gene locus which mediates the response to testosterone should then be regarded as the master of extragonadal sexual developments.

In this paper, I shall attempt to identify the products of these two major regulatory genes of sexual development. The concept is schematically illustrated in Fig. 1.

H-Y ANTIGEN AS THE Y-LINKED TESTIS DETERMINING GENE PRODUCT AND AS THE TESTIS-INDUCING HORMONE

As soon as the migration of primordial germ cells from the yolk sac to the gonadal ridge has completed, the Y-chromosome shows its effect on gonadal organogenesis. In the case of XX individuals, migrant germ cells tend to distribute themselves in the peripheral area of developing gonads. Male germ cells, in sharp contrast, wade right into the central gonadal blastema, and out of the immediate mingling between migrant germ cells and resident somatic elements of the central blastema, seminiferous tubules emerge [1].

The above is the clear indication that the Y-linked testis-determining gene must necessarily specify a plasma membrane protein which is involved in organogenesis through cell–cell recognition and interaction. The Y-linked histocompatibility (H-Y) antigen has indeed been known in mouse since 1955 [2]. As histocompatibility antigens are detected on the surface of viable cells, they are by definition plasma membrane proteins. Development of cytotoxicity tests for H-Y antigen utilizing specific humoral antibody was a major advance [3]. The extreme evolutionary conservation of H-Y antigen soon became evident, for mouse antibody directed against H-Y antigen of the same species recognized the homologous antigen on male cells of most other mammalian species including man [4]. Furthermore, the W-linked histocompatibility antigen present in the heterogametic female sex of the chicken [5] was also detected by mouse H-Y antibody [4]. Such evolutionary conservation indicates the invariant persistence of a specific function, and that specific function almost has to be the organization of heterogametic gonads; testis in mammals and ovaries in birds. The above led us to propose that H-Y antigen is the long-sought after product of the Y-linked testis-determining gene of mammals [6].

The proposed identity between the Y-linked testis-determining gene product and H-Y antigen in mammals can best be tested on exceptional individuals (man and beasts) whose gonadal sex does not agree with

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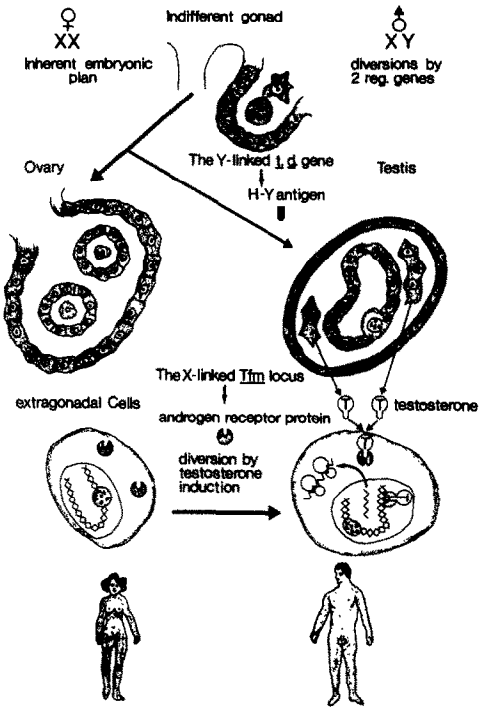

Fig. 1. The mammalian sex determining mechanism dominated by two major regulatory genes is illustrated in a simple schematic manner. The mammalian embryonic plan is innately feminine. The male development is triggered by the Y-linked testis organizing gene which apparently specifies a plasma membrane protein known as H-Y antigen. The Y-induced testis then produces androgen. The nuclear-cytosol androgen-receptor protein ubiquitously presents in the body of both sexes responds to testosterone produced by the testis and activates specific sets of enzyme and protein genes in different target cell types, thus, causing the development of all the extragonadal masculine characteristics. The X-linked *Tfm* locus is believed to specify this androgen-receptor protein. For the sake of clarity, certain important details have been omitted. For example, the XY gonadal cell plasma membrane should possess a specific receptor for H-Y antigen, in addition to H-Y antigen itself. Furthermore, in many androgen-target organs, testosterone is rapidly converted to 5α-dihydrotestosterone, and the androgen-receptor protein shows a higher binding affinity to the latter than to the former.

their apparent sex chromosome constitutions, for under no circumstance should there be a dissociation between the act of testicular organization and the expression of H-Y antigen. A rather extensive series of tests were subsequently performed by S. S. Wachtel and as we had hoped, all individuals that possessed either testes or ovotestes in an apparent absence of the Y-chromosome still typed as H-Y antigen positive, whereas all XY individuals lacking testes but having been endowed with ovaries typed as H-Y antigen negative [7]. Two types of exceptional individuals that proved to be most informative are described in some details below:

1. In the laboratory mouse (*Mus musculus*), XY, *Sxr*/+ male mice transmit an autusomal dominant *Sxr* gene to half of their XX progeny and sex reverse them to males [8]. Not only were these XX, *Sxr*/+ ♂ with testes H-Y antigen positive, but also their XY, *Sx*/*r* sibs typed as H-Y antigen positive. The above clearly indicated that *Sxr*-gene is in reality the testis-determining gene translocated from the Y to an autosome and that this *Sxr*-gene does specify H-Y antigen.

2. In the wood lemming (*Myopus schisticolor*) of Scandinavia, fertile XY ♀ can be found among the progeny of normal XX ♀. Furthermore, when mated to normal XY ♂, the progeny of these XY ♀ include only females: XX ♀ as well as XY ♀ [19]. The above suggests the presence of an X-linked mutant gene in a population which suppresses the expression of the testis-determining gene on the Y, and allows XY zygotes to develop ovaries. XY ♂ of this species typed as H-Y antigen positive, while XX ♀ as well as XY ♀ typed as H-Y antigen negative [7]. Thus, the testis-determining function and H-Y antigen expression were concordantly suppressed in XY ♀. In view of the above, I do believe that our proposal should now be regarded as essentially correct.

The remainder of this section shall be devoted to our observation that H-Y antigen may also serve as the testis-inducing hormone having an extremely short range of effectiveness. Since time immemorial, cattle breeders must have known the curious fact that when cows are born cotwins with bulls, they are almost invariably sterile, showing a somewhat masculinized phenotype. Demonstration in 1916 that chorionic vascular anastomis is a rule between bovine dizygotic twins identified a blood-born influence from a male twin fetus as the cause of freemartinism [10, 11]. The problem has been in identifying the nature of this blood-born influence. Most certainly, it can not be testosterone or any other androgenic steroids. While testosterone is responsible for male development of the accessory glands and ducts and all other secondary sex characteristics, it plays no part in the gonadal sex determination. Yet in the bovine freemartin, testosterone-dependent masculinization is only moderate, typically characterized by the presence of seminal vasicles and an enlarged clitoris. It is her ovary which is virilized due to destruction of the ovarian cortex and subsequent formation of seminiferous tubule-like structure in the ovarian medulla. Indeed, it has been shown that the freemartin gonad produces a substantial amount of androgen [12].

In 1962, it was shown that cellular chimerism in bovine heterosexual twins involves not only hemopoietic organs but also gonads [13]. It was felt that donor cells in the gonad were migrating primordial germ cells that happened to wander into blood circulation. *Sensus stricto*, however, the cell type of donor cells in the gonad is irrelevant. The cellular theory of freemartinism implies that virilization of the freemartin gonad is due to these donor XY cells in its midst [14]. It occurred to us that H-Y antigen might be a key to this cellar theory of freemartinism. What if a minority of donor XY cells is able to disseminate H-Y antigen and able to coat the majority of host XX gonadal cells with it? H-Y antigen-coated XX

gonadal cells might be fooled and might now engage in testiscular organization. Here, one needs only to assume that XX gonadal cells, while lacking the Y-linked H-Y antigen gene, are nevertheless equipped with the specific plasma membrane receptor for H-Y antigen.

Virilization of the freemartin ovary occurs rather late in fetal development [15], thus, we have opted to study three freemartins in 150th to 175th day of gestation that were developing strongly virilized, small testis-like gonads. The absorption test superimposed on the sperm cytotoxicity test as the measure of H-Y antigen is not very sensitive [7]. Thus, only the presence of a substantial amount of H-Y antigen would have given us an unequivacally positive result. It was found that on per cell basis, such strongly virilized freemartin gonad indeed possessed almost as much H-Y antigen as the bull's testis [16]. In passing, we might mention that chimeric hemopoietic organs of these freemartins did not type as impressively H-Y antigen positive as their gonads. It would appear that disseminated H-Y antigen is capable of serving as the testis-inducing hormone The gonadal chimerism as a prerequisite to the transformation of an XX gonad to a testis attests only to its extremely short range of effectiveness.

In the chicken and the duck, double-yolked eggs develop into chimeric dizygation twins. In a heterosexual pair, the ovary of a ZW-female remains unaffected. It is the testis of a ZZ male which becomes feminized to resemble an ovary [17], and the gonadal chimerism has been demonstrated. It would appear that in gonadal organogenesis, cells of whichever the sex that possess H-Y antigen (ZW-cells in birds) exercise the dominance over those which lack H-Y antigen.

The extreme evolutionary conservation of H-Y antigen [4] indicates that its role in the gonadal sex determination has been imposing severe restrictions upon the H-Y antigen gene's freedom to undergo mutational changes. What are the nature of these restrictions? First of all, it can not be too strongly antigenic. Rest the immunization of mammalian females caused by repeated pregnancies would result in fetal death of XY embryos. Indeed, H-Y antigen is a very weak antigen that frustrates investigators attempting to raise effective H-Y antibody. Furthermore, the tolerance to it can be easily induced. Secondly, it appears that on the plasma membrane, H-Y antigen is in association with the major histocompatibility (MHC) antigens [18] and such an association may be a prerequisite to the fulfillment of its assigned function. Needless to say, an association requires the conservation of a binding site or sites. In marmoset monkeys, the chimeric dizygostic twinning is the rule rather than an exception. Yet in the case of heterosexual twins, the female's ovaries are not virilized in the slightest, in spite of an apparent gonadal chimerism [19]. It may be that in primate species, a functional transference of H-Y antigen from XY to XX

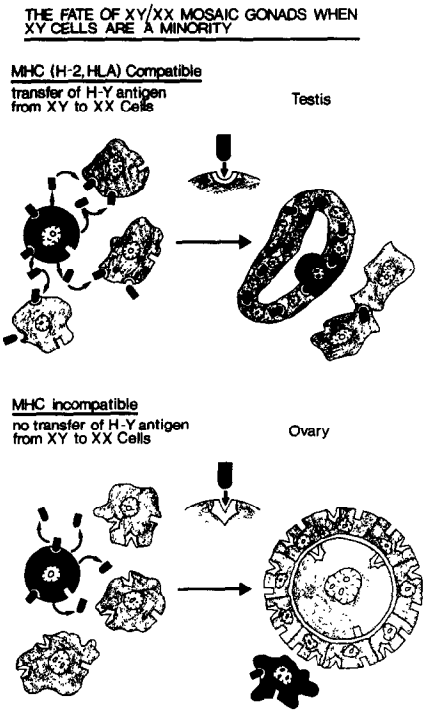

Fig. 2. A minority of XY gonadal cells in the midst of XX gonadal cells appears capable of enticing neighboring XX cells to engage in testicular organization only under the certain circumstances: i.e. the bovine freemartin [6], human XX males which may be cryptic XY/XX mosaics [28] and experimentally produced XY/XX male mice of certain strain combinations [34]. In other circumstances, these mosaic gonads appear to develop as the ovary: i.e. the marmoset monkey chimeric female [19] and experimentally produced XY/XX female mice of other strain combinations [34]. In this scheme, I propose that compatibility (not necessarily genetic identity) at the major histocompatibility antigen (MHC) complex locus is the prerequisite for the successful transference of H-Y antigen (solid black rods) from a XY gonadal cell (drawn solid black) to neighboring XX cells (drawn shaded). As before [16, 28], I assume that XX gonadal cells (germ as well as somatic elements), while lacking H-Y antigen, are equipped nevertheless with specific receptor sites as well as anchorage sites for H-Y antigen (indentations on the cell surface). In view of the demonstrated coexistence of H-Y and MHC (H-2 of the mouse) antigens on the cell surface [18], I believe that the configuration of the anchorage site is determined by MHC antigens (outlined structures on the plasma membrane). When XY cells constitute a majority in the mosaic gonad, it will develop as a testis or ovotestis even in the presence of MHC incompatibility.

gonadal cells does not occur unless the donor and the recipient share the identical MHC genes (Fig. 2).

In concluding this section, I need to emphasize that cardinal fact of gene regulation that any primary regulatory gene by its very definition should not come under the control of any other gene. In short, the production of a primary regulatory gene product should always be constitutive. Indeed, as far as we know, H-Y antigen is expressed by every cell type of the mammalian male body, although its role clearly is confined to testicular organogenesis. We believe that only gonadal cells possess the specific plasma

membrane receptor for H-Y antigen, in addition to H-Y antigen itself. Without the coexisting specific receptor, the presence of H-Y antigen alone in other XY cell types appears redundant and functionally irrelevant.

THE NUCLEAR-CYTOSOL ANDOGEN-RECEPTOR PROTEIN AND THE X-LINKED *Tfm* LOCUS AS THE REGULATORY LOCUS OF SEXUAL PHENOTYPE

While the Y-linked testis-determining gene is of pivotal importance in mammalian sexual development, its role is clearly limited to testicular organization in early embryos. Thus, in spite of the ubiquitous expression of H-Y antigen in all somatic cells [20], no masculine development beyond the formation of testis occurs in XY mice that carry the X-linked *testicular feminization* (*Tfm*) mutation [21]. Accordingly, this *Tfm* locus emerges as the second key regulatory locus that governs all extragonadal sexual developments.

Mammalian embryos have an inherent inclination to develop the feminine phenotype. Therefore, development of male accessory glands and ducts and all other masculine secondary sex characteristics have to be induced by andogen produced by the Y-organized testis. Yet, in the absence of *Tfm* mutations, XX cells are as responsive to andogen as XY cells, for fetal exposure to administered testosterone readily induces the complete set of masculine secondary sex characteristics in XX fetuses, whereas, if deprived of their own internal source of testosterone by early castration, XY fetuses automatically develop the feminine secondary sex characteristics [15].

In man, it has been known for some time that *Tfm* mutation transmitted through heterozygous mothers to half of their XY progeny renders affected XY individuals either totally or partially insensitive to the internally produced as well as externally administered androgen. In view of the extreme evolutionary conservation of mammalian X-chromosomes [22] the subsequently established X-linkage of this mutation in the mouse [21] makes it a virtual certainty that the *Tfm* locus is on the X-chromosome of all other mammalian species including man. Furthermore, there now exists a concensus that *Tfm* caused androgen insensitivity is due to a mutational defect of the nuclear-cytosol andogen-receptor protein in all the species studied: the mouse [23] the rat [24] and man [25].

In this paper, it should then be appropriate to focus our attention on the central question of does the X-linked *Tfm* locus specify the nuclear-cytosol andogen-receptor protein and if so is this the only andogen-receptor locus in the mammalian genome?

1. *The absence of a direct proof of the identity between the Tfm locus and the andogen-receptor locus*

Both in the mouse [23] and the rat [24], a drastic reduction in the amount of andogen-receptor protein to 15–20% of the wild-type level was actually found in target organs of affected *Tfm*/Y. The essentially similar finding has been reported in fibroblasts of human *Tfm*/Y [25]. Needless to say, a mere reduction in the amount does not constitute a desired proof of the identity, for one can argue that *Tfm* is a suppressive mutation of the regulatory locus which controls the activity of andogen-receptor protein loci. An ideal proof of the identity is to show by such means as isoelectric focusing that a *Tfm*/Y andogen-receptor protein differs from its wild-type counterpart by an amino acid substitution. We found this to be a technically difficult, if not impossible, feat. Accordingly, there exists no absolute proof of identity. Nevertheless, indirect evidences in support of the notion that the *Tfm* locus is a structural locus for the andogen-receptor protein abound. Some of the more important ones are discussed below.

2. *The constitutive production of andogen-receptor protein*

Until recently, it was thought that the occurrence of nuclear-cytosol steroid-receptor protein is organ specific, since it is to be found only in target cell types. If this were so, it follows that the steroid-receptor protein locus is not the primary regulatory locus of hormone responses, for its organ specific expression has to be controlled by a regulatory gene belonging to a higher hierarchy. Fortunately, it now appears that most, if not all, of the steroid hormone-receptor proteins are to be found in target and nontarget cell types alike. For example, the andogen-receptor protein has been found in cultured normal male and female human fibroblasts [25], in spite of the fact that no appreciable andogen provoked response can be monitored in these cells. Similarly, mouse fibroblasts in culture have been found to contain not only andogen but also estrogen-receptor proteins [26]. It has also been our experience that the above two species of sex steroid-receptor proteins were found not only in functionally pertinent regions such as the hypothalamus, but also in all other parts of the mouse brain [27]. Although developmental ups and downs in the level of androgen-receptor proteins do occur in different organs, the expression of andogen-receptor protein is essentially ubiquitous as was the expression of H-Y antigen. In as much as the production of androgen-receptor protein is constitutive, there exists no *a priori* need to invoke the presence of a higher regulatory locus for control of the androgen-receptor locus. Being a structural locus for the nuclear-cytosol androgen-receptor protein, the X-linked *Tfm* locus can fulfill the role of being the primary regulatory locus of extragonadal sexual development.

3. *DHT-target organs and TES-target organs*

Certain androgen-target organs can differentiate only in the presence of androgen. Thus, such organs as epididymis, seminal vesicles, prostates and penis are normally found in the male. On the other hand, other androgen-target organs can survive in the

absence of androgen. Accordingly, these organs are found in the male and the female alike; submaxillary salivary glands and kidney of the mouse are the examples.

The former type of androgen-target organs tends to be rich in 5α-reductase which rapidly converts testosterone (TES) to 5α-dihydrotestosterone (DHT). The latter type of androgen-target organ, on the other hand, tends to be poor in this enzyme, while being richly endowed with the second step enzyme which converts 5α-dihydrotestosterone further to 5α-androstane-(3α as well as 3β)-diols. Accordingly, cells in the latter type of androgen-target organs are not normally exposed to 5α-dihydrotestosterone. Not surprisingly, the notion has developed that there ought to be two different kinds of nuclear-cytosol androgen-receptor proteins: 5α-dihydrotestosterone-receptor protein for the former type of target organs and testosterone-receptor protein for the latter type.

In affected *Tfm*/Y, androgen target organs of the former type are entirely missing. It follows then that observed androgen insensitivity of the latter type of target organs has to be due to a mutational deficiency of the testosterone-receptor protein. Thus, an apparent single locus mutation is adversely affecting two independent loci for 5α-dihydrotestosterone-receptor protein and testosterone-receptor protein. Such is certainly incompatible with the proposed identity between the *Tfm* locus and the nuclear-cytosol androgen-receptor locus.

Contrary to the above view, our studies on the mouse has been consistent in finding only one and the same kind of androgen-receptor protein in target organs of the former as well as latter type. This receptor protein always shows a highest binding affinity to 5α-dihydrotestosterone, but it also binds to testosterone with a respectable affinity. Accordingly, in target organs of the latter type that persist in *Tfm*/Y, what is mutationally defective is this what one might call DHT-TES-receptor protein [23, 27]. Undoubtedly, the same mutationally defective DHT-TES-receptor protein caused the developmental failure of the former type of androgen-target organs in *Tfm*/Y.

All in all, I am rather convinced that the X-linked *Tfm* locus is the structural locus for the nuclear-cytosol DHT-TES-receptor protein. How can the single species of androgen-receptor protein ubiquitously expressed in target and nontarget cells alike mediate the specific induction of different sets of proteins and enzymes in divergent target organs? This problem has recently been discussed rather thoroughly [28]. Therefore, there is no need to expand on it further.

4. *Types of defective mutations that affect the Tfm locus*

Needless to say, a few different kinds of mutations affecting the androgen-receptor locus can cause androgen insensitivity. An apparently drastic reduction in the amount such as observed in *Tfm*/Y mice and rats as well as in some human *Tfm*/Y [23–25] must be due to either a decreased synthesis or an accelerated break down caused by an amino acid substitution, and such a mutation is likely to alter the receptor's kinetic property as well. In fact, we have some suggestive evidence that the mouse *Tfm*/Y receptor-protein exhibits an altered kinetic property [27]. Partial or imcomplete androgen-insensitivity shown by some human *Tfm*/Y patients, on the other hand, is probably caused by a mutated receptor's reduced binding affinity to its androgen ligands. There can also be a what the late Gordon Tomkins termed "nuclear translocation minus mutation" [29]. A mutated receptor in this case should be normal in the cytosol with regard to both the amount and its binding affinity to steroid ligands, but steroid-bound receptors are unable to move into the nucleus. The subtlest mutation of all should be the type that alters the receptor's allosteric property. In the case of the wild-type receptor, the act of binding to an inducing steroid hormone causes the creation of an acceptor binding site *via* an allosteric effect, and presumably it is through this acceptor binding site that steroid-bound receptor proteins associate with specific sites of the genetic apparatus in a nucleus, thus, causing the induction of specific gene products. If a mutation alters the receptor's allosteric property, the creation of a defunct acceptor binding site would result. Because many proteins possess rather indiscriminate binding affinities to the nuclear chromatin, I doubt very much whether one can distinguish such a subtly mutated, androgen insensitive receptor from the wild-type receptor. Nevertheless I am confident that in time all these predictable types of mutations of the X-linked *Tfm* locus shall be found among *Tfm*/Y individuals of man and beasts.

AN ALTERNATE AUTOSOMAL ANDROGEN-RECEPTOR LOCUS FOR MALE GERM CELLS?

Demands imposed by the maturation process of male germ cells are clearly very different from those of male somatic cells. Witness the requirement for a lower temperature for example. It has been suggested that during latter stages of the maturation process, the X-chromosome needs to be totally inactivated in male germ cells [30]. Indeed, in one rodent species, the X-chromosome is eliminated from male germ cells at the stage of differentiation to definitive spermatogonia [31]. It follows then that were male germ cells to remain androgen dependent as most people suppose, they can no longer rely on the X-linked *Tfm*/Y locus. Either they have to receive the X-linked form of the nuclear-cytosol DHT-TES-receptor protein form sertoli cells or they have to activate an alternate autosomal isozyme locus for the androgen-receptor protein, if such an alternate locus exists. It is worth noting that in the case of one household enzyme, phosphoglycerate kinase, all the somatic cells utilize the X-linked form, only in male germ cells

of many mammalian species has an alternate autosomally inherited isozyme been found [32].

The recent demonstration by M. F. Lyon's group that *Tfm*/Y mouse male germ cells, if placed in the normal XY host, can complete the maturation process to produce fertile sperm clearly established the independence of male germ cells from the X-linked *Tfm* locus [33]. The above most likely means that contrary to a popular belief, the male germ cell maturation process is androgen independent. An alternative explanation is that male germ cells utilize an alternate autosomal locus for their androgen-receptor protein. This alternate possibility is currently being studied in my laboratory.

Acknowledgements—This work was supported by NIH Contract NO1-CB-33907 and grants 1R01AG00042 and HD CA 07975.

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DISCUSSION

Naftolin. This is very interesting data. I would like to raise two points. (1) There is a true difference between aggressive behaviour and male sexual behaviour. A good example of that is in the red deer, which Roger Short has castrated out of season and then implanted with pellets of estradiol. These stags now will copulate with the females in the herd, in fact they regain complete copulatory behaviour. But they will not go into the herd: they will stand outside the herd and when a doe comes out they will copulate with her. Based on 2 different forms of behaviour your supposition is very reasonable. (2) Some time ago you compounded the idea of a simplified action of genes and proposed that the (allosteric) events attending steroid binding involved transcriptional effects and translational events. Do you still believe that's true?

Ohno. My view is that *bona fide* transcriptional control would be rather difficult in mammals, for the simple reason that our genome contains too much DNA, most of which is nongenic: 3,500,000,000 base pairs/haploid. So that were they to attempt to seek out the proper *operator* DNA base sequence of a specific set of structural genes they control, such attempts by steroid-bound receptors shall be frustrated by nonspecific bindings to nonsensical DNA sequences. On this purely theoretical ground, I tend to favour the posttranscriptional control in which steroid-bound receptors seek out specific *messenger* RNA precursors in the nucleus to stabilize and process them. Unfortunately, oft observed increase in specific cytoplasmic *messenger* RNAs following the steroid hormone treatment can be caused either by the transcriptional control *sensus stricto* or by the type of post-transcriptional control mentioned above.

McEwen. I would like to make sure I understand the cataloguing of the behaviors in *Tfm* mice which are or aren't organized by estrogen treatment. You said that these animals show acyclicity?

Ohno. If ovarian tissues are transplanted to adult *Tfm/Y* mice following the castration, most of them exhibit the constant estrus syndrome. It would appear that so far as this facet of masculinization of the central nervous system is concerned, a small amount of estradiol converted *in situ* from neonatal androgen was sufficient for the task in *Tfm/Y* brain.

McEwen. Secondly, you showed a masculinization of aggressive behavior. Did you mention copulatory behavior and lordosis behavior?

Ohno. In our stock of mice, normal females, when they are in estrus, mount each other. So mounting behavior *per se* can not be regarded as the true masculine behavior.

McEwen. So it is not a sexually dimorphic characteristic.

Ohno. No, but aggression toward an intruding male is definitely the male specific behavior. While the neonatal estradiol treatment can induce this masculine behavior in normal females, we have thus far failed to elicit this behavior in *Tfm/Y* by the same treatment. It could be that this aspect of neonatal masculinization of the central nervous system is androgen dependent.

McEwen. What about lordosis behavior? That is, would neonatally estrogenized *Tfm*'s show less lordosis than untreated *Tfm*'s, even under replacement therapy with estrogen plus progesterone after ovariectomy?

Ohno. There is something very peculiar about these *Tfm/Y*. Castrated *Tfm/Y* so treated with estradiol and progesterone do not seem to attract male mice. After an overnight with males, we have never found vaginal plug in *Tfm/Y*. Instead, they are often attacked by males. It may be that they do not smell right.

Grumbach. Dr. Ohno is obviously a very parsimonious scientist in hypothesis and has done so much to clarify our understanding of not only sex differentiation and also sex determination which he did not have time to discuss. I'd like to ask you of a situation which has come up recently, namely an *X*-linked pedigree in which there was nuclear binding this from Dr. Migen's group at Hotkins and you may have seen the article in the proceedings of the National Academy of Science and that is they found nuclear binding of DHT–cytoplasm complex. I should say they found dihydrotestosterone bound to nuclei, presumably through the cytoplasmic receptor. Would this imply that in this situation without activation, with the phenotype feminizing testis syndrome would this be one of your allosteric abnormalities rather than something wrong with the DNA.

Ohno. Dr. Grumbach refers to the finding reported on a particular group of human testicular feminized patients by Amrheim *et al.* It seems that this group was characterized by the possession of apparently normal androgen-receptor protein. First of all, such finding does not prove the existence of another androgen-receptor locus in addition to the *Tfm* locus in the human genome. Rather such finding seems to suggest that in the presence of the wild-type androgen-receptor, a mutational deficiency of another *X*-linked locus can render the entire somatic cell population of the body totally nonresponsive to androgen. However, I consider this possibility as extremely remote. I recall that of several classes of *in vitro* generated hydrocortisone-resistant mutant clones of lymphoma cells recovered by the Gordon Tomkin's group, there was a class which was characterized by the presence of apparently normal hydrocortisone-receptor. Yet, Ulrich Gehring tells me that when this class was hybridized with the common receptor deficient class, resulting somatic hybrids remained hydrocortisone resistant. Thus, genetic complementation tests have proven that apparently normal hydrocortisone-receptors were in fact functionally defective. Another point that I like to make on this opportunity is that these studies on human testicular feminized patients were done on cultured fibroblasts which can hardly be considered as androgen-targets. It would appear that the production of androgen-receptor protein is constitutive, so that it is expressed in target and nontarget cell types alike. Nevertheless, the nuclear binding of androgen-bound receptors in a nontarget cell type can not prove the functional normalcy of an androgen-receptor in question.

Grumbach. So you feel this would be an allosteric defect in receptor.

Dörner. Dr. Ohno, as you know, a testicular feminizing like syndrome can also be produced in animal experiments by giving antiandrogen, for example cyproterone acetate, during critical differentiation periods. Furthermore, we have observed (together with Dr. R. Witkowski) monozygotic human twins: One of these twins was a normal male and the other one showed the testicular feminizing syndrome. Would you therefore agree that some cases with testicular feminizing syndrome may also be based on persistent modifications, i.e. permanent nonresponsiveness or at least decreased responsiveness to androgens caused by a temporary androgen deficiency during the intra-uterine life?

Ohno. As you know, mutational defects affecting a number of androgen synthesizing enzymes can give the feminine phenotype to *XY* males. Such defects, however, should readily be remedied by the administration of exogenous androgen throughout the fetal and postpubertal

life. Exogenous androgen, on the contrary, is of no help to testicularfeminized mutants.

DeMoor. Dr. Ohno, going back to the one androgen receptor hypothesis, how can you explain the cases described by Walsh, Imperator and other authors where 5α-reductase is absent and where there is a partial feminisation. Testosterone seems to take over the role of DHT only in part.

Ohno. We can detect only one kind of androgen-receptor in divergent organs of male and female mice, and this receptor shows the highest binding affinity to DHT but also shows a respectable binding affinity to testosterone itself. The mouse kidney, for example, shows a very low 5α-reductase activity, whereas it very rapidly converts DHT to androstanediols. Thus, the androgen-receptor in these kidney cells must normally be responding to testosterone and not to DHT. Yet as far as exogenously administered androgens are concerned, these kidney cells respond better to DHT than testosterone. My belief, therefore, is that if a circulating testosterone concentration is enhanced by continuous exogenous administration of testosterone, prostates and other androgen target organs of 5α-reductase deficient human *XY* fetuses would show the normal masculine development. In this connection, I like to mention that 5α-reductase of many androgen-target organs is normally inducible by androgen, thus, it is under the control of the *Tfm* locus.