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Sex determination in mice: Y and chromosome 17 interactions

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Summary

Mice provide material for studies of Y-chromosomal and autosomal sequences involved in sex determination. Eicher and coworkers have identified four subregions in the mouse Y chromosome, one of which corresponds to the *Sxr* fragment. This fragment demonstrates that only a small portion of the Y is necessary for male sex determination. The mouse Y chromosome also shows variants: the BALB/cWt Y chromosome, which causes nondisjunction of the Y in some germ cells leading to XO and XYY cells and resulting in many infertile true hermaphrodites; the Y^{Dom}, a wild-type chromosome which can result in sex reversal on a C57BL/6J background; and Y-chromosomal variants detected with Y-derived genomic DNA clones among inbred strains. Two different autosomal loci affecting sex differentiation have been identified in the mouse by Eicher and coworkers. The first of these has not been mapped to a particular chromosome and has been designated *Tda*-1 (Testis-determining autosomal-1). This is the locus in C57BL/6J mice at which animals must be homozygous in order to develop as true hermaphrodites or sex-reversed animals in the presence of Y^{Dom}. The other locus has been identified on proximal chromosome 17. This locus also caused hermaphrodites on the C57BL/6J background and it is most easily interpreted as a locus deleted in T^{hp}. It is located in a region on chromosome 17 containing other genes or DNA sequences that may be related to sex determination. These include both the *Hye* (histocompatibility Y expression) locus that affects the amount of male-specific antigen detected by serological and cell-mediated assays and a concentration of Bkm sequences. Despite the Y and chromosomal 17 localizations of Bkm sequences, there is no evidence that transcripts from these are involved in sex determination: RNA hybridizing to sense and anti-sense Bkm clones can be detected in day-14 fetal gonads of both sexes.

Key words: sex determination, mouse, Y chromosome, Bkm sequences.

Introduction

Sex determination or sexual differentiation

The term 'determination' is used in embryology to indicate irreversible commitment to a particular developmental pathway. We have chosen 'determination' for the title of this review so that the subject matter will be clear. That is, we are only concerned with the early sexual developmental stages and not the later ones (involving the Müllerian inhibition factor, testosterone, testosterone receptors, etc.) for which the term 'sexual differentiation' has usually been used.

Chromosomal sex determination

Sex is not firmly fixed in many lower vertebrates. Several species of fish show changes of sex from female to male or male to female under environmental conditions where excesses of particular sexes will maximize reproductive efficiency (Warner, Robertson & Leigh, 1975). Removal of the single ovary in some species of birds will allow the undeveloped second gonad to develop as a testis (Mittwoch, 1971). Other vertebrates may have their sex readily altered by environmental agents. Sexual determination in amphibians can be controlled by addition of sex steroids to the water in which the embryos are developing (Witschi & Dale, 1962) while the sexes of

many species of reptiles can be influenced by the temperature at which their eggs are incubated (Ferguson & Joanen, 1982; Mirosovsky, 1980). This flexibility in sex determination is also reflected in flexibility of the genetic organization of sex determination. Genes determining testicular and ovarian development predate chromosomal sex determination in evolution. Autosomal loci, rather than sex chromosomes, are usually involved in fish sex determination, while other fish, reptiles and amphibia may have either sex with heterogametic sex chromosomes [platyfish have both in one population (Bull & Charnov, 1977)] while birds are heterogametic in the female and mammals are heterogametic in the male (Ohno, 1967). It seems clear that there is a selective advantage for the occurrence of sex chromosomes. It is possible that the selective force is the advantage of maintaining an intact block of sex differentiation genes. This would be most easily understood if at least one of the sex chromosomes carried a block of genes involved in sex determination or sex differentiation. However, to date, the male-determining properties of the Y chromosome seem to be determined by a rather small amount of DNA sequence. This review of sex determination in mice will be selective in presenting Y-chromosomal and chromosome 17 variants which affect sex determination. An excellent, more general review is provided by Eicher & Washburn (1986).

Y-chromosomal variants and sex determination

The mouse Y chromosome has been extensively studied and other papers in this volume present new findings. Eicher and coworkers (Eicher, Phillips & Washburn, 1983) have used cloned sequences and translocation chromosomes to identify four subregions in the mouse Y chromosome: (1) the centromere; (2) a region containing testes-determining genes, transplantation H-Y antigen and Bkm, sex-related sequences; (3) a region containing genetic information for sperm motility and also sequences recognized by retroviral probes and an *Eco*RI, 1·8 kb mouse Y-specific clone and (4) an X-pairing and X-recombination segment. This review focuses on Y-chromosomal variants that affect sex determination.

The BALB/c Y chromosome and hermaphroditism

In 1956, Hollander and associates described a high incidence of hermaphroditic (they used the term gynandromorphic) mice in the BALB/Gw inbred strain (Hollander, Gowen & Stadler, 1956). Most of the hermaphrodites appeared in an X-irradiation experiment but no significant difference between treated and control groups could be demonstrated. The authors were impressed that family group, treatment, age of the mother and size of litter were not significant factors and that the sex of each side of the body appeared to be random. Interestingly, in light of temperature effects on reptilian sexual differentiation, they thought that warmer seasons might increase the incidence of this 'sexual reversal'. A major reason why follow up of this early observation occurred is the clear cut variation among different inbred strains. Nine other inbred strains were studied and were not found to have the hermaphrodites (Hollander *et al.* 1956). Whitten and associates at the Jackson laboratory have provided most of the further data on this phenomenon which has been clearly shown to be attributed to the BALB/c Y chromosome. The more recent studies have used the BALB/cWt strain. Breeding studies that introduced the BALB/cWt Y chromosome onto other genetic backgrounds demonstrated that the tendency for hermaphroditism was a property of the Y chromosome; segregating genetic factors interacting with the Y chromosome were identified in recombinant inbred lines (Beamer, Whitten & Eicher, 1978). The hermaphrodites were usually found to contain ovotestes with location of ovarian tissue predominantly at the gonad poles; there was no difference between left and right sides with regard to gonad type (Whitten, Beamer & Byskov, 1979). Careful karyotypic studies disclosed that these hermaphrodites were chromosomal mosaics of XO/XY or XO/XY/XYY chromosomal constitution (Eicher, Beamer, Washburn & Whitten, 1980). The XO germ cells are presumably selectively eliminated in the spermatogenic epithelium (Levy & Burgoyne, 1986). This pattern of mosaicism is most compatible with Y-chromosomal nondisjunction during mitosis. Thus, it is apparent that the probable role of the BALB/c Y chromosome in this hermaphroditism is to determine a tendency towards mitotic nondisjunction.

The Sxr *Y fragment*

The *Sxr* condition is the result of an apparently autosomal dominant mutation. This sex-limited condition causes XX mice to appear as phenotypic males. These 'males' have external and internal male organs normal in every respect except for the existence of complete azoospermia (Cattanach, 1975). *Sxr*,XY mice are fertile males whose only phenotypic aberration is slightly reduced testes size. The possibility that the *Sxr* condition is due to an inherited Y-chromosomal fragment was first raised by Winsor and collaborators (Winsor, Ferguson-Smith & Shire, 1978). They detected a putative Y-chromosomal fragment at an autosomal location in *Sxr*,XO and *Sxr*,XY mouse spermatocytes at diakinesis. We performed cytological studies of mitotic preparations, using both

whole-mount electron microscopy and light microscopy of silver-stained preparations and detected a supernumerary Y-chromosomal fragment in *Sxr*,XY mice which was not seen in normal XY litter mates (co-isogenic controls; Shapiro, Erickson, Lewis & Tres, 1982). The chromosomal segment was identified as being of Y origin by its pairing with the homologous paracentromeric region of the Y chromosome during late zygotene–pachytene and by the structural features of its axial core. Studies with Bkm sequences also pointed in the direction of the presence of a portion of the Y chromosome in *Sxr* mice. The Bkm sequences are arranged in a sex-specific pattern in mice as seen in Southern blots and preferentially hybridize to the proximal region of the Y chromosome with *in situ* cytogenetic analyses. Hybridization of these sequences to Southern blots of *Sxr*,XX DNA reveal an arrangement of these sequences similar to that of normal murine male DNA (Singh, Purdom & Jones, 1980). *In situ* chromosomal hybridization with the Bkm sequences demonstrated that XX,*Sxr* mice show a concentration of hybridizing sequences on the putative X chromosome while XY,*Sxr* carrier males had two small clumps of hybridizing sequences which were interpreted as being due to a pair of variant Y chromosomes (Singh & Jones, 1982). Hansmann (1982) suggested that the apparent pair of Y's were the separate chromatids of the Y and that the correct interpretation was a Y chromosome with an extra block of Bkm sequences. This is the currently accepted interpretation and it fits well with our current understanding of the pseudoautosomal region and the exchanges that occur between the X and Y (Burgoyne, 1986). We had demonstrated increased serological H-Y in *Sxr*,XY carrier males (Shapiro *et al.* 1982). The discovery of *Sxr'* which still determines maleness but not transplantation H-Y, indicated a definite disassociation of transplantation H-Y from the testes-determining factor (McLaren *et al.* 1984 and this symposium). Interestingly, the extra material presumably present in *Sxr* but not in *Sxr'* is required for spermatogenesis in XO,*Sxr* males which might indicate that transplantation H-Y is necessary for normal spermatogenesis (Burgoyne, Levy & McLaren, 1986).

The Y^{DOM} chromosome

While introducing an alpha-thalassemia mutant onto the C57BL/6J background, Eicher and associates discovered that the Y chromosome being introduced was the cause of hermaphroditism (Eicher, Washburn, Whitney & Morrow, 1982). They traced this chromosome to the *Mus poschiavinus* species which is currently classified as a subspecies of *Mus musculus domesticus* (*Mus domesticus*). This apparent incompatibility between the Y^{DOM} (Y from *Mus domesticus*) chromosome and X or autosomal responder genes was found with Y chromosomes of several other *Mus domesticus* strains but only on the genetic background of the C57BL/6J strain. Thus, this seems to be an example of chromosomal incompatibility which follows Haldane's rule (Haldane, 1922). Haldane's rule states that 'when in the F_1 offspring of two different animal races, one sex is absent, rare, or sterile, that sex is the heterozygous (heterogametic) sex'. However, in *Drosophila* it seems that Haldane's rule is due to a incompatibility between the two sex chromosomes rather than between sex chromosomes and autosomes (Coyne, 1985) while an autosomal locus seems to be responsible for the incompatibility between the C57BL/6J strain and Y^{DOM} (Eicher & Washburn, 1983). Interestingly, although the standard laboratory inbred stocks, including C57BL/6J, are most like *Mus domesticus* for a variety of protein polymorphisms, their Y chromosomes seem mostly to have been derived from the *Mus musculus musculus* species (Y^{MUS}). Lamar & Palmer (1984) first found Y-chromosomal restriction fragment length polymorphisms that separated the standard inbred strains into a common class, including C57BL/6J, and a minor class including SJL and AKR. Bishop *et al.* 1985 showed that the two classes represented a common class with a *Mus musculus* Y chromosome while the SJL strain was unusual in having a *Mus domesticus* Y chromosome. Thus, C57BL/6J begins as autosomal^{DOM} and X^{DOM} but with a Y^{MUS}! One supposes that adaptation to a possible Y incompatibility has occurred. Also, the Y^{DOM} chromosomes which have caused hermaphroditism on the C57BL/6J background were all derived from mice captured in Italy. Although the Italian mice are considered to belong to the same species as standard inbred lines (*Mus domesticus*) they are quite distinct for many biochemical loci. This is relevant to the possibility that this incompatibility is an example of Haldane's rule which is couched in terms of 'races'. If indeed the Y^{DOM} is from a different 'race' of *Mus domesticus*, then the hermaphroditism that develops in the C57BL/6J mice with this particular Y^{DOM} chromosome can be seen as reflecting variation in Y chromosomes among mice from different geographical regions or as a change in autosomal or X-linked loci to adapt to the Y^{MUS} standardly present.

Chromosome 17 variants and sex determination

Several autosomal loci affecting sex differentiation have been identified in the mouse. The first of these has not been mapped to a particular chromosome and

has been designated *Tda*-1 (Testis-determining autosomal-1). This is the locus in C57BL/6J mice at which animals must be homozygous in order to develop as true hermaphrodites or sex-reversed animals in the presence of the Y^{DOM} (Eicher & Washburn, 1983). We will focus on variation related to sex determination which maps to chromosome 17.

Hye, *histocompatibility H-Y expression*

Although it is now clear that transplantation H-Y is not a sex-determining substance, the question is not yet answered for serological H-Y or, as it is sometimes called, MSA (male-specific antigen). The serologically detected antigen(s) was initially assumed to be the same H-Y as detected by transplantation because of the similar dependence on maleness (Goldberg *et al.* 1971; Scheid, Boyse, Carswell & Old, 1972). The histocompatibility Y expression (*Hye*) locus was defined in a immunological test measuring a cell-meditated response: the popliteal node enlargement assay (Kralová & Démant, 1976). The effects of *Hye* on defining 'antigen strength' in this assay map somewhat nearer to the *T* locus than to the *H-2* complex on chromosome 17 (Kralová & Lengerová, 1979). We found that the effects of *Hye* can also be detected by quantitative immunoabsorption (Shapiro & Erickson, 1984). Thus, a chromosome 17 locus affects the amount of H-Y detected by serological or cell-mediated methods in a parallel fashion. If this is not a fortuitous coincidence, it may be the result of quantitative variation in a molecule that can be detected by both serological and histocompatible methods. If so, *Hye* would modify the expression of transplantation H-Y mapped to the *Sxr* fragment of the Y chromosome and lost in *Sxr'*. Alternatively, the locus defined as *Hye* could have an effect on maleness through other mechanisms with secondary effects on detectable amounts of male specific antigens.

T *deletions and hermaphroditism*

We have already discussed the hermaphroditism that occurs on the C57BL/6J inbred strain with certain Y chromosomes. Perhaps the C57BL/6J strain's sex-determining mechanisms are 'weaker' since other genetic manipulations causing hermaphroditism have been detected using this inbred strain. Washburn & Eicher (1983) reported a partial to complete sex reversal with the T^{hp} deletion on this and other genetic backgrounds. They suggested that this result might be due to a primary sex-determining locus (*Tas*) closely linked to, or a part of, the *T*-complex. Hermaphroditism with another *T* deletion, T^{Orl}, has also been noted on the C57BL/6J background (Eicher & Washburn, 1986). Thus, it is perhaps easier to think of the effect of *T* deletions as the result of the deletion of a sequence or sequences involved in sex determination than as a locus linked to these *T* mutations. It is conceivable that the sequences deleted could be those for the *Hye* locus. It is also conceivable that the sequences deleted are the Bkm sequences which show a regional concentration on this portion of chromosome 17.

Regional localization of Bkm sequences on chromosome 17

Our interest in the possible localization of Bkm sequences on chromosome 17 came from earlier studies of the morphology of the *Sxr* fragment reviewed above (Shapiro *et al.* 1982). We observed a Y fragment, Y^{f}, in *Sxr*,XY carrier males. It frequently paired with the Y and seemed attached to, or was close to, an autosome. In retrospect, this Y fragment must be attached to the Y (the pseudoautosomal region was not appreciated at that time) but with a thin interconnecting fragment, as in the human fragile X. During pachytene, the Y^{f} is either free or associated with an autosome within the length range of chromosomes 15, 16 and 17. Since mouse chromosomes 12, 15, 16, 18 and 19 have a nucleolar-organizing function and no nucleolus is associated with the autosome close to the Y^{f}, it was possible that chromosome 17 associated with Y^{f}. In order to test this assumption, we used a Robertsonian chromosome, Rb7 (Rb [16;17] 7BnR) as a marker of chromosome 17. Silver-stained spermatocytes at pachytene display: (1) synaptonemal complexes in the autosomes, (2) paired X and Y chromosomes and (3) the Rb7-forming part of the condensed chromatin mass of the sexual bivalent (Fig. 1). Because Bkm sequences known to be present in *Sxr* might provide a basis for pairing to an autosome, we studied the regional localization of Bkm sequences in mouse chromosomes using the *Drosophila* clone, pCS316 obtained from K. Jones (Kiel-Metzger & Erickson, 1984). We found two regions of increased concentration of Bkm sequences on the X chromosome and regional concentrations on two autosomes, one on proximal 17 (Fig. 2). It is intriguing that this regional localization of Bkm sequences near the major histocompatibility complex may have been maintained in evolution. A regional localization is conserved between mouse chromosome 17 and human chromosome 6 (Kiel-Metzger, Warren, Wilson & Erickson, 1985). The regional localization is very close to *H-2* in mice but separated by some distance from HLA in humans. This pattern of close linkage of the MHC on mouse chromosome 17 but with a more distant localization to q21 on human chromosome 6 is also found for mitochondrial superoxide dismutase.

We have recently been provided with four putative Bkm-positive cosmid clones from proximal chromosome 17 by A. Craig and H. Lehrach. Three were

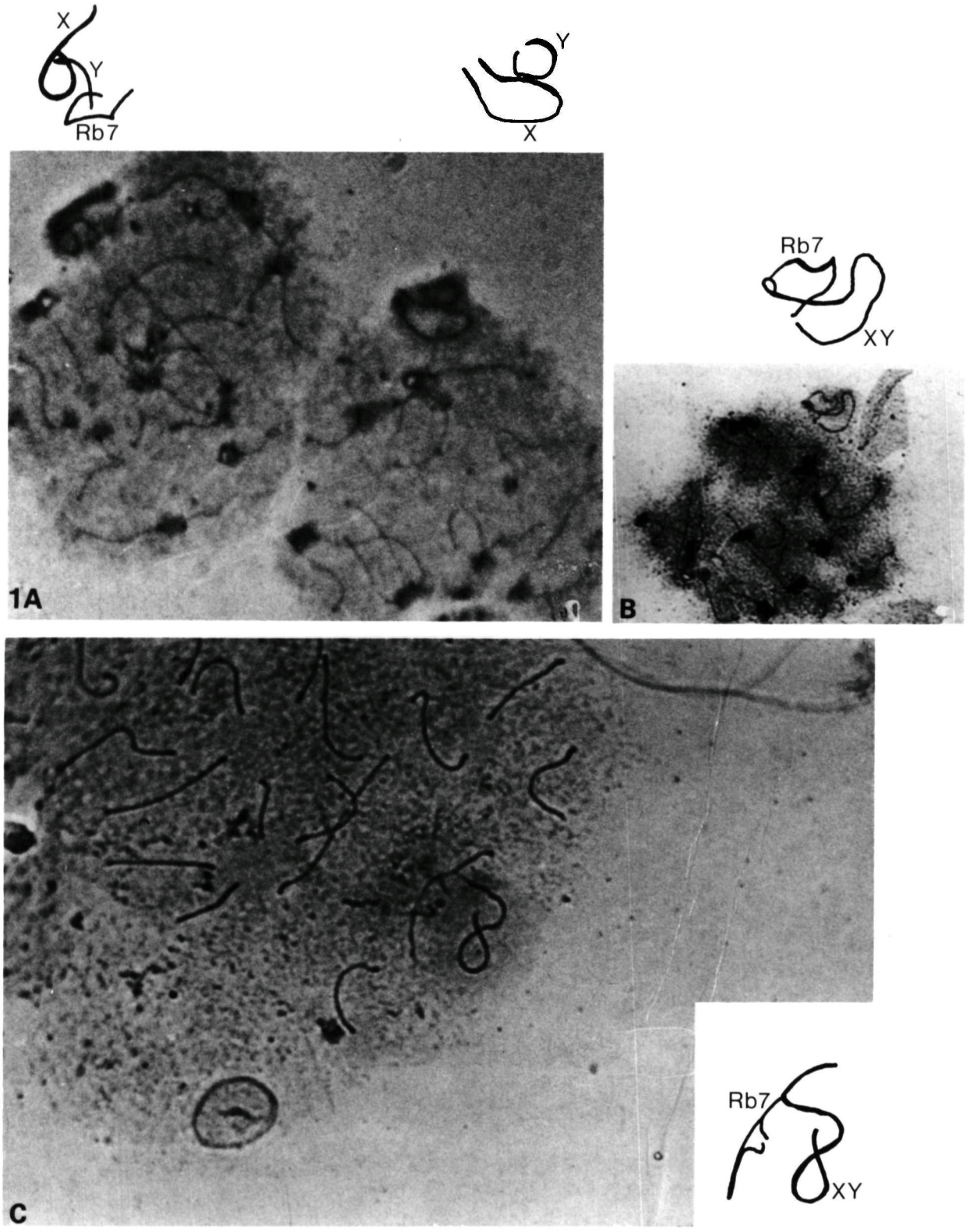

Fig. 1. Silver-stained spermatocytes of *Sxr*,Rb7 compound heterozygotes (see Shapiro *et al.* 1982 for Methods). In A two cells are seen. The one on the left shows an association of the XY bivalent with the Rb7 metacentric while they are not associated in the cell on the right. In B and C, the terminalized XY bivalent is associated with Rb7; in C, Rb7 is partially desynapsed.

confirmed as Bkm-positive by their hybridization as dot blots to the synthetic olido-deoxynucleotide $(GATA)_5$. Southern hybridization of the restricted cosmids shows interspersed Bkm-positive and non-Bkm-hybridizing DNA. Despite this confirmation of the localization of Bkm sequences to proximal 17, we remain skeptical about a role for Bkm sequences in sex determination.

Although there have been previous studies of Bkm-related transcripts, they have been limited to adult tissues (Singh, Phillips & Jones, 1984; Schafer *et al.* 1986). It seemed more logical to us to search for sex-specific transcripts in fetal gonads since this would be closer to the time of sex determination and there ought to be a greater likelihood of finding such transcripts if they are present. In order to perform such studies, the riboprobe constructs previously described (Erickson *et al.* 1987), were used in Northern analyses of total RNA prepared from various tissues at various stages of mouse development. RNA was prepared from brain, liver and gonad of 14-day embryos, newborn animals and adults by the guanidinium isothiocyanate and caesium chloride method. These blots have been analysed with both sense and anti-sense Bkm probes at various levels of stringency. At lower levels of stringency, cross hybridization to 28 and 18 S ribosomal RNAs was observed as well as a few smaller, putative transcripts in female ovary and

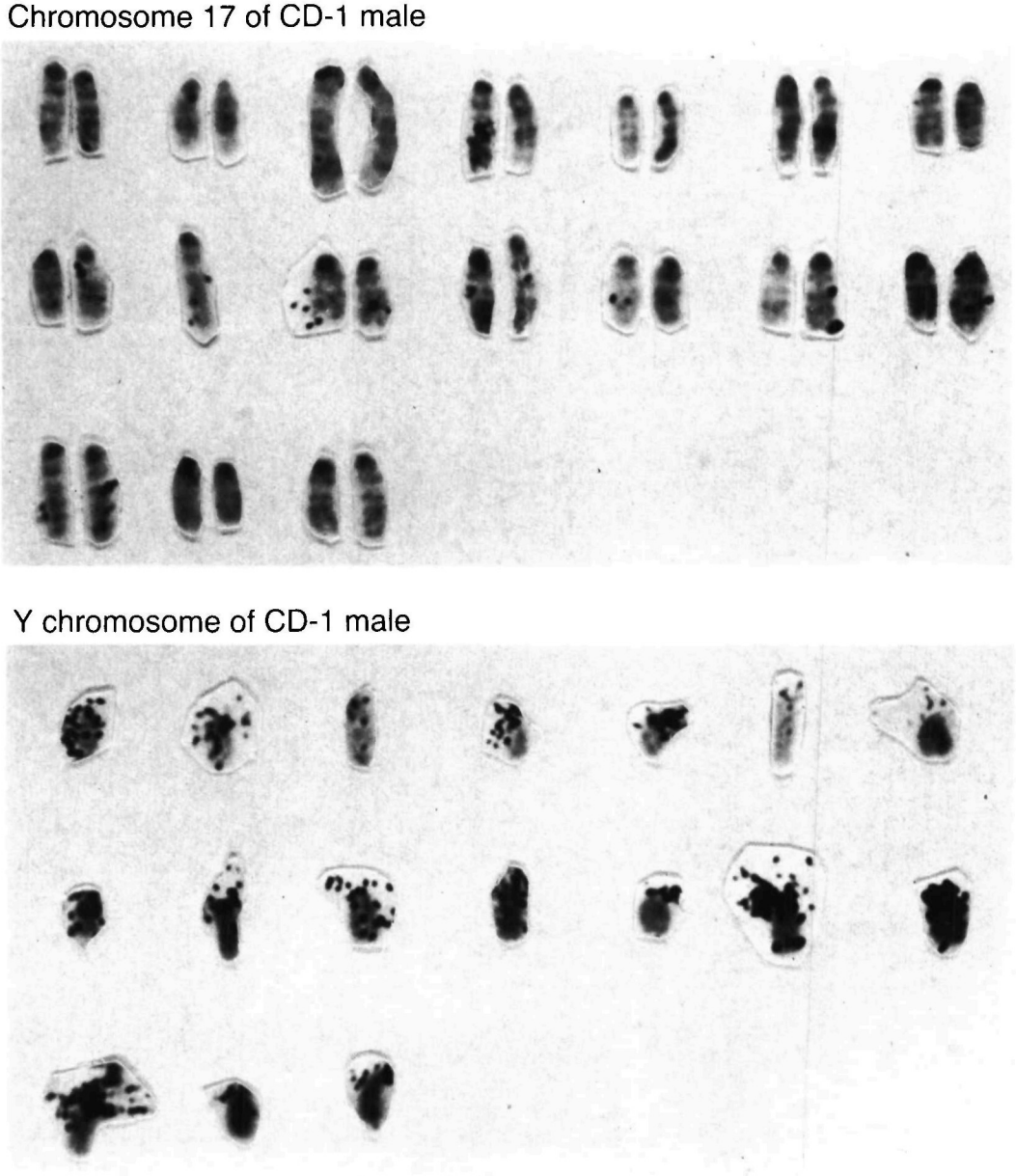

Fig. 2. *In situ* hybridization of Bkm-containing pCS316 with chromosome 17 and the Y of the random-bred, CD1 line (see Kiel-Metzger & Erickson, 1984 for Methods). The larger amount of hybridization to the Y may be compared to the regional localization on chromosome 17.

male brain in adult but not in newborns or feti (E. J. Durbin & R. P. Erickson, unpublished data). The other transcript which was not mere artifactual binding to ribosomal RNA was a 12 kb transcript seen in all adult tissues with both the sense and anti-sense probe. However, when poly A^+ RNA was prepared, this putative transcript was no longer detected. Thus, using the highly sensitive riboprobe method, we have not been able to detect sex- or tissue-specific Bkm-related transcripts.

Conclusion

It is clear that sex determination in mice involves more than Y chromosomal sequences. This should not be surprising since any such complex developmental pathway ought to require multiple genes and there is not much evidence for multiple sex determination genes clustered on the Y chromosome. Our research, and that of others, has particularly focused on chromosome 17 as containing relevant autosomal loci. It is not yet clear whether there really is an unusual concentration of loci involved in sex determination on chromosome 17 or whether, because this is the location of the *t*-complex, mutations in this region have been sufficiently studied to note their effects on sex determination. However, it is interesting that the proximal region of chromosome 17 has a number of loci affecting spermatogenesis – the obvious later function of the male gonad. This includes the transmission-ratio-distorting properties (Erickson, Lewis & Butley, 1981; Lyon, 1984) and the male sterility properties (Hammerberg, 1982; Lyon, 1986) of the *t*-complex. In addition, the hybrid sterility locus (*Hst*) is on proximal chromosome 17 with an effect on spermatogenesis not related to *t*-complex alleles (Forejt, 1981). Only further study will determine whether or not the sex determination variants found on chromosome 17 are related to, or interact with, these other variations on chromosome 17 related to male sexual function. However, it is interesting to note that C57BL/6J is not only an unusual strain for sex determination, it is the strain with the highest incidence of aberrant spermiogenesis described to date (Hillman & Nadijcka, 1978).

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