Chromosoma (Berl) (1989) 97:301–306

CHROMOSOMA

Characterization of GATA/GACA-related sequences on proximal chromosome 17 of the mouse

E.J. Durbin[1], R.P. Erickson[1], and A. Craig[2]

[1] Departments of Human Genetics and Pediatrics, University of Michigan Medical School, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA

[2] Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, UK

Abstract. Autosomal loci have long been thouht to have a role in sex determination of mice. We studied the localization of GATA/GACA repeats on chromosome 17 in regard to the possibility of their involvement in sex determination. We performed in situ hybridizations on chromosome 17s carrying the Hairpain tail (T^{hp}) deletion of the *T* locus since this deletion has been associated with sex reversal and hermaphroditism. We did not detect a significant decrease in the amount of hybridization of GATA/GACA repeats to the T^{hp} deletion. In addition, three Bkm-positive cosmids from proximal chromosome 17 did not contain sequences deleted in T^{hp} or T^{Orl} and a fetal testes cDNA probe did not hybridize to the cosmid sequences. Although we confirmed the localization of Bkm-related sequences on chromosome 17, we were not able to relate GATA/GACA sequences on chromosome 17 to sex determination in mice.

Introduction

There has been great interest in the possible role of chromosome 17 loci in sex determination of mice for several reasons. Firstly, two deletions of proximal chromosome 17 have been found to cause problems with sex determination. Hairpin tail (T^{hp}) is an allele at the brachyury (*T*) locus (Johnson 1974). When $T^{hp}/+$ males are repeatedly backcrossed to C57BL/6J females, many hermaphrodites as well as sex-reversed animals are produced (Eicher and Washburn 1986). Another deletion of a brachyury allele, *T*-Orleans (T^{Orl}) also causes sex reversal when $T^{Orl}/+$ females are repeatedly backcrossed to C57BL/6J males with a Y chromosome of *Mus musculus domesticus* (Y^{DOM}) origin (Eicher and Washburn 1986). In addition to the association of the *T* region of mouse chromosome 17 with sex determination, other genes with effects on the male gonad are found on the same chromosome. The quaking (*qk*) mutation disrupts normal germ cell differentiation (Bennett et al. 1971) whereas the *Hst-1* locus causes male sterility in hybrids from certain laboratory and wild mouse strains (Forejt and Ivanyi 1975). In addition, the Histocompatibility Y-expression (*Hye*) gene controlling amounts of transplantation H-Y antigen (Kralova and Lengerova 1979) and serological H-Y antigen (male specific antigen, MSA; Shapiro and Erickson 1984) map near *T*. Three sterility loci have also been mapped to chromosome 17 (Lyon and Mason 1977; Hammerberg 1982). Finally chromosome 17 is of interest because of a regional localization of Bkm sequences in the proximal region (Kiel-Metzger and Erickson 1984). Bkm sequences contain many GATA tetranucleotide repeats as well as some GACA repeats (Epplen et al. 1982). These sequences have been thought by some authors to be involved in sex determination although their role, if any, has not been proven conclusively.

We have found three Bkm-positive cosmid clones from the proximal region of chromosome 17. We herein characterize these cosmid clones in terms of Bkm and non-Bkm sequences and test if they contain sequences from chromosome 17 associated with sex determination in mice. We have also used the T^{hp} deletion to see if Bkm sequences are lost. If, in fact, they are, one might argue that Bkm-related sequences are involved in some aspect of sex determination. We did not find an effect of the two deletions (T^{hf} and T^{Orl}) on Bkm sequences, either by in situ hybridization or in regards to the cosmids, but we confirmed the localization of Bkm sequences to proximal chromosome 17.

Materials and methods

The T^{hp} and T^{Orl} mice used in this study were obtained from the laboratory of Dr. Vernon Bode. The Rb(1,17) lem mice maintained in this laboratory were originally obtained from Dr. J. Forejt, Czechoslovakian Academy of Sciences, Prague, Czechoslovakia.

Clones. Three Bkm-positive cosmid clones were obtained from the proximal part of chromosome 17 of the mouse. Cosmids T459/A and W459/A were recovered from 129 and t^{w5} libraries by in vivo recombination using sequences derived by microdissection of chromosome 17 (Röhme et al. 1984). Cosmid C81 was derived from a cell-line library constructed from MDMD (B) BI (David Housman) which carries mouse chromosome 17 on an MDCK background.

The actin probe, pAl, a 2.0 kb PstI insert in pGEM-4 (Promega), was constructed by Dr. Bevilacqua of this laboratory. The mouse LDH-X cDNA clone, pPM459 (Fujimoto et al. 1984; Tanaka and Fujimoto 1984) is from our laboratory, whereas the pancreatic amylase cDNA (Gumucio et al. 1985) was obtained from Dr. M. Meisler. The Drosophila Bkm probe, pCS 316, was the gift of Dr. K. Jones.

Hybridization. Conditions similar to those of Schafer et al. (1986) were used for hybridization with the synthetic oli-

Offprint requests to: R. Erickson

gonucleotide $(GATA)_5$ except that the temperature was 42° C. This probe was end-labeled with T4 polynucleotide kinase and [γ-^{32}P] ATP (Maniatis et al. 1982). Hybridization with pCS316, total mouse, or cosmid DNA was carried out at 65° C for 16–20 h in a solution containing 1 M NaCl, 1% SDS, and 100 µg/ml sheared salmon sperm DNA. These DNA probes were labeled to high specific activity using [α-^{32}P]-CTP and random priming (Feinberg and Vogelstein 1984).

In order to identify unique sequence portions of the mouse chromosome 17 cosmids that contained Bkm-hybridizing sequences, cosmids C81, T459/A and W459/A were digested with EcoRI and electrophoresed on a 0.8% agarose gel (Maniatis et al. 1982). DNA transfer was performed with Hybond-N (Amersham) and hybridization was carried out as above using the $(GATA)_5$ oligonucleotide. The filter was stripped in 0.4 M NaOH and rehybridized to total mouse DNA. Fragments of each cosmid which did not hybridize to either probe were eluted from a 1% agarose gel. Each fragment was used to probe slot blots containing various mouse DNAs. The mouse DNA filters were stripped as before in 0.4 M NaOH and rehybridized to pCS316 (a Bkm-related repetitive sequence) in the same way. The autoradiograms were scanned with the Zeineh Soft Laser Scanning Densitometer with the densitometry program by Biomed. The amount of hybridization was compared between the different DNAs to see if a decrease in hybridization of any of the fragments was observed in T^{hp} or T^{Orl}. In addition samples of T^{hp}, T^{Ori}, C57B16/J and random-bred mouse DNA were digested with EcoRI and TaqI, run on a 0.9% agarose gel (Maniatis et al. 1982) and transferred top Hybond-N. Each of the cosmid fragments from before was hybridized as above to the filter. The autoradiograms were scaned as above.

Fetal testes cDNA studies. Total cellular RNA was isolated from the testes of 14 day post-coitum fetuses by the guanidinium isothiocyanata-cesium chloride method (Chirgwin et al. 1979). Poly(A)$^+$ RNA was prepared using oligo(dT) cellulose column chromatography (Maniatis et al. 1982). A radiolabeled cDNA first strand probe was synthesized with reverse transcriptase (Kraus et al. 1986). This cDNA probe was used to hybridize to the chromosome 17 cosmids digested with EcoRI (see above).

In situ hybridization. Metaphase chromosome spreads were prepared from short-tail and normal-tail progeny at the age of 6 weeks from the cross of Rb1,17 X+/T^{hp}. The normal-tail offspring were the control. Slides were treated with RNase, heat denatured and hybridized according to the protocol of Kiel-Metzger and Erickson (1984) modified from Harper and Saunders (1981). The *Drosophila* Bkm insert, pCS316, was nick-translated with [^{3}H]dATP, dCTP, dTTP to a specific activity of 1×10^7 cpm/µg DNA. After 16 h of hybridization at 37° C, slides were initially washed in 2 X SSC, 50% formamide at 39° C followed by less stringent washes and passage through an ethanol series (Harper and Saunders 1981). (1 X SSC is 0.15 M NaCl, 0.015 M sodium in rate.) Autoradiography was carried out according to Harper and Saunders (1981) with staining in 0.25% Wrights stain diluted 1:3 with 0.06 M phosphate buffer (Chandler and Yunis 1978); the method was modified to include destaining in 95% ethanol, 1% HCl for 15 s, and absolute methanol for 15 s and restaining (Kiel-Metzger and Erickson 1984). This procedure was repeated until satisfactory banding was achieved. Chromosomes were observed with a 100× oil immersion lens and grains counted that were touching at least one chromatid. Over 150 spreads were analyzed.

Results

The synthetic oligonucleotide $(GATA)_5$ was hybridized to EcoRI-digested cosmid DNAs. We found both Bkm and non-Bkm sequences in all three cosmids (Fig. 1). The cosmid clone T459/A contained four Bkm-positive fragments of approximately 20, 5.6, 3.8 and 3.4 kb. Three Bkm-positive fragments of 5.8, 5.4, and 4.8 kb were observed in cosmid clone W459/A whereas C81 had positive fragments of approximately 15, 6.6, 5.5, and 5.0 kb. DNA blot hybridization with total mouse DNA as a probe gave information about other repetitive sequences in the non-Bkm DNA. Restriction fragments which were present in the ethidium bromide stained gel (Fig. 2) but which were not visible when hybridized to either probe were considered to be non-hybridizing fragments. Cosmid T459/A had one fragment of approximately 4.1 kb, W459/A had two fragments of approximately 5.6 and 5.2 kb, and C81 contained four fragments of approximately 3.5, 2.8, 2.3, and 1.5 kb which hybridized to neither probe. The largest non-hybridizing fragment from each cosmid was eluted and used as a low copy number probe to see whether that particular sequence was deleted in T^{hp} or T^{Orl} mice. Dosage analysis by densitometry for the fragments from C81 and W459/A did not show the 50% decrease in hybridization expected if the fragments were deleted from the T^{hp} or T^{Orl} chromosome 17 (Table 1). Surprisingly, there was an increase in hybridization to T^{hp} DNA with the fragment from T459/A.

The possibility that any of these cosmid DNA sequences was transcribed in fetal testes was studied using a total cDNA probe from 14 day post-coitum fetal testes. This radiolabeled cDNA was used as a probe for hybridization to the EcoRI-digested cosmid DNA. It failed to hybridize to any of the chromosome 17 cosmid sequences even though we observed hybridization to LDH-X and amylase DNAs (Fig. 2). Thus, these cosmids do not encode any sequences expressed at reasonable abundance in fetal testes.

We looked for a decrease in hybridization of Bkm sequences to chromosome 17 deletions in the *T* region by in situ hybridization. Inasmuch as Bkm sequences have been found to be localized to this region of chromosome 17 (Kiel-Metzger and Erickson 1984), a decrease in the amount of hybridization in these heterozygous deletion mice would support the hypothesis that these GATA/GACA repeats mapped near to loci involved in, or were themselves involved in, sex determination. Localizations to both the X and Y chromosomes as well as to two autosomes were observed for the T^{hp}/Rb(1,17) and control mice (Fig. 3). Over 40% of the X chromosomes and 55% of the Y chromosomes showed hybridization to the Bkm probe. This was expected since an earlier study reported heavy localization of Bkm-related sequences on the sex chromosomes of mice (Singh and Jones 1982). Chromosomes 4 and 17 also showed an increased amount of hybridization when compared with the other autosomes. In this study, 16% of chromosomes 4 were labeled (Fig. 3). These results confirm the localization of Bkm-related sequences on chromosomes 4 and 17 (Kiel-Metzger and Erickson 1984). Although there

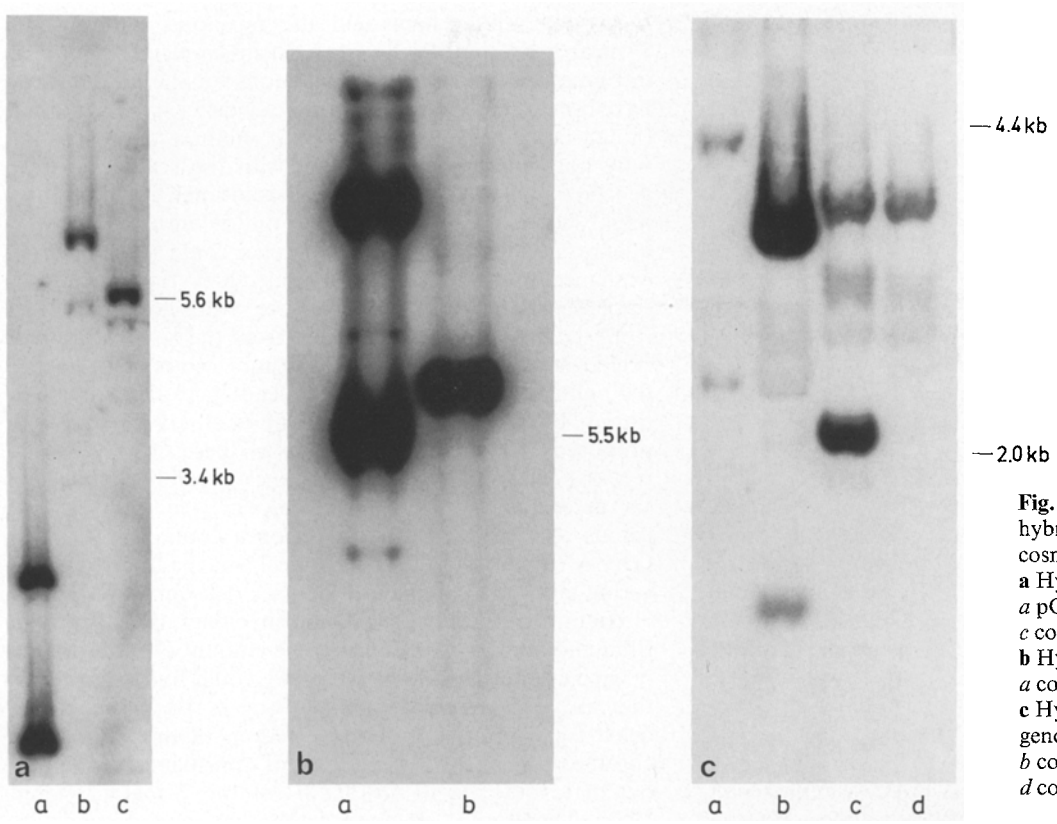

Fig. 1a–c. DNA blot hybridization of EcoRI-digested cosmid DNAs.
a Hybridization of $(GATA)_5$ with *a* pCS316, *b* cosmid T459/A, *c* cosmid W459/A.
b Hybridization of $(GATA)_5$ to: *a* cosmid C81, *b* pCS316.
c Hybridization of total mouse genomic DNA to: *a* pCS316, *b* cosmid C81, *c* cosmid T459/A, *d* cosmid W459/A

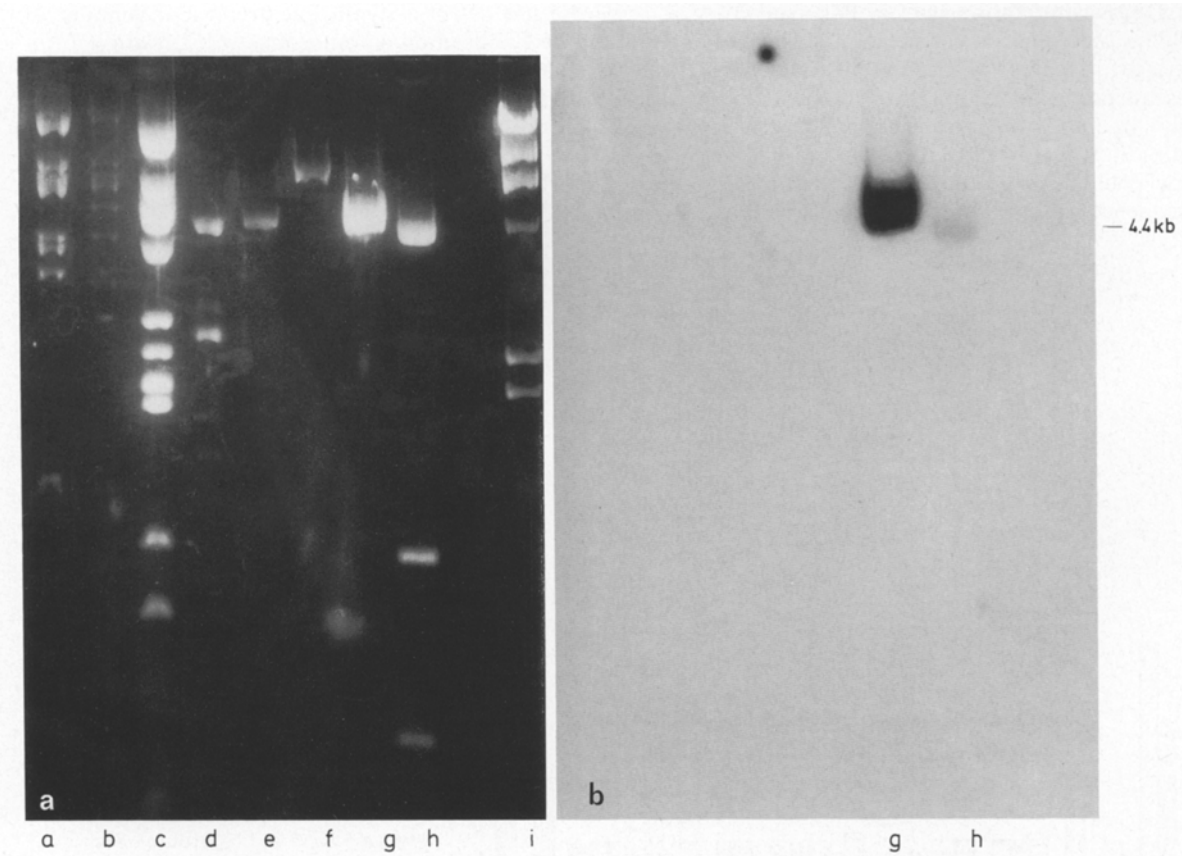

Fig. 2a, b. Hybridization of EcoRI-digested cosmid DNAs. **a** Ethidium bromide stained gel. **b** Hybridization with radiolabeled cDNA from 14 day fetal testes. *a* Cosmid T459/A, *b* cosmid W459/A, *c* cosmid C81, *d* pCS316, *e* pBR322, *f* actin probe, *g* LDH-X cDNA, *h* amylase cDNA, *i* λ HindIII. Fragment sizes for λ HindIII from top in kb: 23, 9.4, 6.6, 4.4, 2.3, 2.0

Table 1. Ratio of hybridization of cosmid DNA to hybridization of repetitive DNA for mouse DNAs to determine dosage[a]

Mouse DNA	Cosmid		
	C81[b]	W459/A[c]	T459/A[d]
Control[e]	1.0±0.2[f]	1.2±0.3	1.6±0.4
T^{Orl}	1.2±0.1	1.2±0.3	1.8±0.4
T^{hp}	1.3±0.2	1.4±0.4	2.9±0.3

[a] Ratios calculated as area determined by densitometry and standardized to 1.0 for the lowest value (control DNA with cosmid C81 4.1 kb fragment as probe)
[b] 4.1 kb EcoRI fragment
[c] 5.6 kb EcoRI fragment
[d] 3.5 kb EcoRI fragment
[e] Includes +/+ and B1/6 mouse strains
[f] Mean ±standard error

was a trend toward a decrease in hybridization to the T^{hp} chromosome 17 when compared with the Robertsonian chromosome 17, this was a decrease of only 14% for the T^{hp} chromosome 17 and it was not statistically significant ($P>0.1$).

Discussion

The question of a function for GATA/GACA simple repetitive sequences has still not been answered although recent evidence casts doubt on their role as the primary sex determiner in mammals (Page et al. 1987). The probable cloning of the mammalian testis-determining gene by Page and coworkers, while ruling out Y chromosome Bkm sequences as the male sex trigger, does not negate the potential role of Bkm or other sequences at other points in the sex determination pathway. In fact, it requires any putative role of Bkm to be with autosomal or sex-linked loci. For mice such autosomal loci could be on chromosome 17. The localization of Bkm sequences in close proximity to the mouse Y sex-determining locus and also on mouse chromosome 17 near genes potentially involved in sex determination is intriguing. Several potential functions for the Bkm repeats have been discussed but no positive evidence has been found (Singh et al. 1984; Chandra 1985). If these sequences are only nonfunctionally associated with the sex-determining gene(s) on the chromosome, their evolutionary conservation might be hard to explain. For the Y chromosomal sequences, their evolutionary persistence could be more easily explained by non-homologous pairing of the Y chromosome during meiosis such that these sequences are not lost from the Y as readily as they are from the X or autosomes (Platt and Dewey 1987). These authors also report evidence for multiple evolutionary events leading to the accumulation of Bkm sequences as Levinson et al. (1985) have also proposed. Whatever the function of these GATA/GACA repeats, their association with genes possibly involved in sex determination on chromosome 17 (see Introduction) led us to characterize cosmid clones containing GATA/GACA repeats from chromosome 17 in the hope of uncovering autosomal loci involved in sex determination.

In our characterization of cosmid clones, three had both Bkm and non-Bkm sequences. The amount of Bkm present in each cosmid varied. However, we could find no evidence that any of the fragments from any of the cosmids was deleted in T^{hp} or T^{Orl}. These deletions of the T region of chromosome 17 involve a region of approximately 3–5 cM out of a total size of 15–20 cM for the T region (Forejt 1981). Therefore it seems probable that these cosmids are not from the deleted region and as such are uninformative about T^{hp} and T^{Orl}.

We did not detect a significant decrease in amount of Bkm on the T^{hp} deletion chromosome 17 by in situ hybridization. As mentioned earlier, T^{hp} is a large deletion which spans the T^{Orl} deletion such that if we could not find a significant decrease in hybridization to T^{hp}, we would not expect one for T^{Orl}. The deletion in T^{Orl} includes the T region markers, *RP17* and *T119*, with duplication of *Tme* and *Tcp-1* (Rogers 1986) which are also all deleted in the

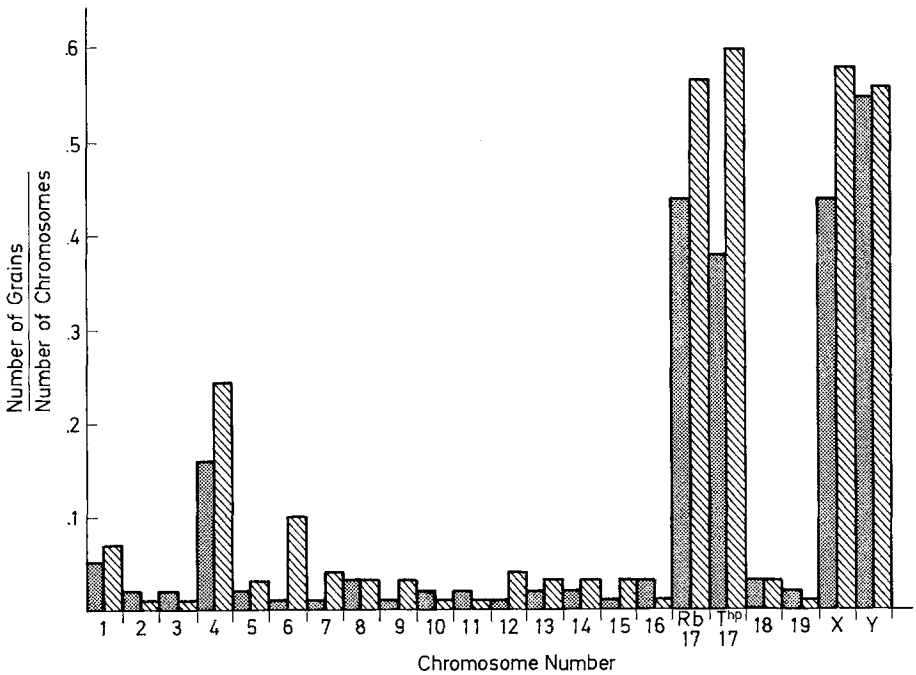

Fig. 3. Histogram of frequencies of hybridization of pCS316 DNA to T^{hp} (*shaded boxes*) and control (*diagonal lines*) chromosomes

T^{hp} mouse (Bennett 1975). As shown by Kiel-Metzger and Erickson (1984) two regions of chromosome 17 appear to be preferentially hybridized by the Bkm-related probe pCS316. The first region nearest the centromere in region 17A3-C could be in the area deleted by T^{hp} and T^{Orl} although Lyon et al. (1979) map T^{hp} to 17A2. This would make the Bkm sequences more distal to the centromere and as such our lack of evidence for a decrease in hybridization of Bkm to the T^{hp} chromosome 17 could be easily explained. The relative resolution of grains in this technique also causes difficulty in assigning the exact band location on chromosome 17. Therefore, since the Bkm-related sequences are most likely outside the region deleted by either T^{Orl} or T^{hp}, they are probably not involved in sex determination related to the *Tas* locus assigned to chromosome 17 by Eicher and Washburn (1986).

Although there is strong evidence from electron microscopic and genetic studies for involvement of chromosome 17 with the Y chromosome in sex determination (Erickson et al. 1987; Eicher and Washburn 1986) the role of chromosome 17 loci has not been proven conclusively. Kasahara et al. (1987) have reported the random cloning of a testis-specific gene from the proximal portion of chromosome 17. Using mouse testis cDNA as a probe they were able to find a 2.0 kb transcript present only in testes. However no function was reported for this gene. Our cosmids do not appear to have sequences which code for a testes-specific transcript since our attempts to hybridize a radiolabeled fetal testes cDNA to the cosmid DNA proved negative. Again, perhaps our cosmids contain sequences nearby but outside of the region of chromosome 17 involved in sex determination. One interesting result of our study with the fetal testes cDNA as a probe involved our finding of hybridization to LDH-X and amylase clones. LDH-X is not detected post-natally until spermatocytes are present (Meistrich et al. 1977) while amylase expression has only previously been detected in salivary glands, pancreas, and, at very low levels in liver (Osborn et al. 1987). These cDNA clones contain poly(A) which could have hybridized to poly(T) in the fetal testes cDNA probe. One other possibility is that these genes are expressed for a short period of time in the fetal testes but we consider this to be unlikely.

Although we were able to confirm localization of Bkm-related sequences to proximal chromosome 17 of mice, we found no evidence that these autosomal sequences are involved in sex determination. In addition, three Bkm-positive cosmids from this chromosomal region do not appear to contain sequences deleted in either T^{hp} or T^{Orl} mice. From this we conclude that although there is other evidence for chromosome 17 involvement in some aspects of murine sex determination, our results do not indicate that the Bkm-related sequences function in this regard.

Acknowledgements. The authors thank Drs. Vera Verga and Michal Witt for critically reading this manuscript and Ann Mogan for her assistance in preparing this manuscript. The authors are also grateful for the assistance of Dr. Hans Lehrach. This research was supported by NIH grants HD20670 and Training Grant T32 HD07274.

References

Bennett D (1975) The T-locus of the mouse. Cell 6:441–454

Bennett WI, Gall AM, Southard JL, Sidman RL (1971) Abnormal spermiogenesis in quaking, a myelin-deficient mutant mouse. Biol Reprod 5:30–58

Chandler ME, Yunis JJ (1978) A high resolution in situ hybridization technique for the direct visualization of labeled G-banded early metaphase and prophase chromosomes. Cytogenet Cell Genet 22:352–356

Chandra HS (1985) Sex determination: a hypothesis based on non-coding DNA. Proc Natl Acad Sci USA 82:1165–1169

Chirgwin JM, Przybla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299

Eicher EM, Washburn LL (1986) Genetic control of primary sex determination in mice. Annu Rev Genet 20:327–360

Epplen JT, McCarrey JR, Suton S, Ohno S (1982) Base sequence of a cloned snake W-chromosome DNA fragment and identification of a male-specific putative mRNA in the mouse. Proc Natl Acad Sci USA 79:3798–3802

Erickson RP, Durbin EJ, Tres LL (1987) Sex determination in mice: Y and chromosome 17 interactions. Development Suppl 101:25–32

Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Addendum. Anal Biochem 137:266–267

Forejt J (1981) Hybrid sterility gene located in the T/t-H-2 supergene on chromosome 17. In: Reisfeld RA, Ferrone S (eds) Current trends in histocompatibility. Plenum Press, New York, pp 103–131

Forejt J, Ivanyi P (1975) Genetic studies of male sterility of hybrids between laboratory and wild mice (Mus musculus L.). Genet Res 24:189–206

Fujimoto H, Erickson RP, Quinto M, Rosenberg MP (1984) Post-meiotic transcription in mouse testes detected with spermatid cDNA clones. Biosci Rep 4:1037–1044

Gumucio D, Wiebauer K, Dranginis A, Samuelson L, Treisman L, Caldwell R, Antonucci T, Meisler M (1985) Evolution of the amylase multigene family: YBR/Ki mice express a pancreatic amylase gene which is silent in other strains. J Biol Chem 260:13483–13489

Hammerberg C (1982) The influence of T^{Orl} upon male fertility in t-bearing mice. Genet Res 37:71–77

Harper ME, Sounders GF (1981) Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma 83:431–439

Johnson DR (1974) Hairpin-tail: a case of post-reductional gene action in the mouse egg? Genetics 76:795–805

Kasahara M, Figueroa F, Klein J (1987) Random cloning of genes from mouse chromosome 17. Proc Natl Acad Sci USA 84:3325–3328

Kiel-Metzger K, Erickson RP (1984) Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 in mice. Nature 310:579–581

Kralóva J, Lengerová A (1979) H-Y antigen: genetic control of the expression as detected by host-versus-graft popliteal lymph node enlargement assay maps between the T and H-2 complexes. J Immunogenet 6:429–438

Kraus JP, Williamson CL, Firgaira FA, Yang-Feng TL, Munke M, Francke U, Rosenberg LE (1986) Cloning and screening with nanogram amounts of immunopurified mRNAs: cDNA cloning and chromosomal mapping of cystathionine β-synthase and the β subunit of propionyl-CoA carboxylase. Proc Natl Acad Sci USA 83:2047–2051

Levinson G, Marsh JL, Epplen JT (1985) Cross-hybridizing snake satellite, Drosophila, and mouse DNA sequences may have arisen independently. Mol Biol Evol 2:494–504

Lyon MF, Mason I (1977) Information on the nature of t-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. Genet Res 29:255–266

Lyon MF, Evans EP, Jarvis SE, Sayers I (1979) t-haplotypes of the mouse may involve a change in intercalary DNA. Nature 279:38–42

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Meistrich ML, Trostle PK, Frapart M, Erickson RP (1977) Biosynthesis and localization of lactate dehydrogenase X in pachytene spermatocytes and spermatids of mouse testes. Devel Biol 60:428–441

Osborn L, Rosenberg MP, Keller SA, Meisler MH (1987) Tissue-specific and insulin-dependent expression of a pancreatic amylase gene in transgenic mice. Mol Cell Biol 7:326–334

Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack J, McGillivary B, de la Chapelle A, Brown LG (1987) The sex-determining region of the human Y chromosome encodes a finger protein. Cell 51:1091–1104

Platt THK, Dewey MJ (1987) Multiple forms of male-specific simple repetitive sequences in the genus Mus. J Mol Evol 25:201–206

Rogers JH (1986) The mouse t complex is composed of two separate inversions. Trends Genet 2:145

Röhme D, Fox H, Herrmann B, Frischauf A-M, Edström JE, Mains P, Silver LM, Lehrach H (1984) Molecular clones of the mouse t complex derived from microdissected metaphase chromosomes. Cell 36:783–788

Schafer R, Ali S, Epplen JT (1986) The organization of the evolutionary conserved GATA/GACA repeats in the mouse genome. Chromosoma 93:502–510

Shapiro M, Erickson RP (1984) Genetic effects on quantitative variation in serologically detected H-Y antigen. J Reprod Immunol 6:197–210

Singh L, Jones KW (1982) Sex reversal in the mouse (Mus musculus) is caused by a recurrent nonreciprocal crossover involving the X and aberrant Y chromosome. Cell 28:205–216

Singh L, Phillips C, Jones KW (1984) The conserved nucleotide sequences of Bkm, which define Sxr in the mouse, are transcribed. Cell 36:111–120

Tanaka S, Fujimoto H (1986) A postmeiotically expressed clone encodes lactate dehydrogenase isozyme X. Biochem Biophys Res Commun 136:760–766

Received May 3, 1888 / in revised form August 2, 1988
Accepted by S. Wolff