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A Deletion Map of the Human Y Chromosome Based on DNA Hybridization

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SUMMARY

The genomes of 27 individuals (19 XX males, two XX hermaphrodites, and six persons with microscopically detectable anomalies of the Y chromosome) were analyzed by hybridization for the presence or absence of 23 Y-specific DNA restriction fragments. Y-specific DNA was detected in 12 of the XX males and in all six individuals with microscopic anomalies. The results are consistent with each of these individuals carrying a single contiguous portion of the Y chromosome; that is, the results suggest a deletion map of the Y chromosome, in which each of the 23 Y-specific restriction fragments tested can be assigned to one of seven intervals. We have established the polarity of this map with respect to the long and short arms of the Y chromosome. On the short arm, there is a large cluster of sequences homologous to the X chromosome. The testis determinant(s) map to one of the intervals on the short arm.

INTRODUCTION

In mammals, gonadal sex—whether one has testes or ovaries—is determined by the presence or absence of the Y chromosome. Regardless of the number of X chromosomes per cell, mammalian embryos with a Y chromosome develop

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testes, while those without a Y chromosome develop ovaries [1]. Therefore, it is generally assumed that one or more genes on the Y chromosome trigger the gonad to differentiate into a testis rather than an ovary.

Many structural anomalies of the human Y chromosome have been detected by light microscopy of stained mitotic chromosomes. Inferences as to the regional location of the testis determinant(s) on the human Y chromosome have been drawn from correlations of these abnormal karyotypes with the sex phenotypes [2, 3]. However, the precision of such chromosome-banding studies is limited, and there is often considerable uncertainty as to the structure of such abnormal Y chromosomes. For these reasons, debate continues as to whether the testis determinant(s) map to the short arm, centromeric region, or long arm of the Y.

Cloned Y-chromosomal DNA sequences represent a new tool for the analysis of the Y chromosome and its role in testis determination. Several types of Y-chromosomal DNA sequences have been identified. Among the Y-specific repeated sequences that have been described, the best studied are those that appear as discernible 2.1- and 3.4-kilobase (kb) bands on *Hae*III restriction digests of male genomic DNA [4]. Some single-copy Y sequences are highly homologous to single-copy sequences on the X chromosome [5–9]. Other single-copy Y sequences exhibit weaker homology to the X chromosome [10, 11] and/or to autosomes [12]. Finally, as judged by hybridization, an apparent minority of single-copy Y sequences show no homology to any other chromosome [8, 12].

Human "XX males" are sterile males whose karyotype is 46,XX. About one in 20,000 males is an XX male, and the vast majority of cases occur sporadically [13]. It has been hypothesized that XX males carry a small, male-determining portion of the Y chromosome, undetected by conventional chromosome-banding studies [14]. Indeed, using cloned Y sequences as DNA hybridization probes, it has been shown that Y-specific DNA is present in some XX males and that these XX males are heterogeneous with respect to the amount of Y DNA in their genomes [15, 16].

Our study extends these studies of XX males. We describe the systematic use of an expanded collection of cloned Y-DNA hybridization probes to analyze numerous structural anomalies of the human Y chromosome. For several of these Y anomalies, light microscopic studies were also informative. The results allow us to construct a physical or deletion map of the human Y chromosome that is composed of seven intervals, which is oriented with respect to the long and short arms of the chromosome and in which the testis determinant(s) is assigned to one interval on the short arm.

MATERIALS AND METHODS

Patients Studied

The patients we have studied are listed in table 1. The phenotypes and karyotypes of many of these individuals have been reported elsewhere (references in table 1). Most of the individuals studied were karyotyped during the course of a medical evaluation because of infertility and/or small testes. (In table 1, the individuals who are fertile are

TABLE 1

KARYOTYPES AND PHENOTYPES OF PATIENTS STUDIED

Case	Identification	Karyotype	Phenotype	Reference
1	LGL208	46,XX	True hermaphrodite	Case 5 in [17]
2	CHM002	46,XX	True hermaphrodite*	...
3	CHM003	46,XX	Male*	...
4	LGL200	46,XX	Male	Case 4 in [17]
5	LGL203	46,XX	Male	Case 6 in [17]
6	...	46,XX	Male	Case 2 in [15]
7	PAR019	46,XX	Male	...
8	PAR020	46,XX	Male	...
9	CHM006	46,XX	Male	...
10	CON101	46,XX	Male	...
11	...	46,XX	Male	Case 3 in [15]
12	GRB027	46,XX	Male	...
13	GM2670	46,XX	Male†	Case 2 in [18]
14	GM2626	46,XX	Male†	Case 1 in [18]
15	LGL163	46,XX	Male	[19]
16	LGL197	46,XX	Male	Case 3 in [17]
17	CHM005	46,XX	Male	...
18	...	46,XX	Male	Case 1 in [15]
19	...	46,XX	Male	Case 4 in [15]
20	LGL105	46,XX	Male	Case 7 in [17]
21	LGL115	46,XX	Male†	[20]
22	CHM004	46,XYq−	Male	...
23	SL	46,XYq−	Male	...
24	LL92	46,XYq−	Male	...
25	CHM018	46,XYq−	Male	...
26	CHM007	46,XYq−	Male‡	...
27	CHM008	46,XX,t(Y;15)	Female§	Case b in [38]

* CHM002 and CHM003 are siblings.
† GM2670 and GM2626 are second cousins and are remotely related to LGL115.
‡ Fertile, normal male with a short Y chromosome lacking quinacrine-bright region.
§ Apparently normal female with a 46,XX,der(15),t(Y;15) (q12;p11) karyotype; has a fertile sister with the same karyotype.

specifically identified. All those not so indicated are sterile.) Nineteen males (testicular tissue only) and two true hermaphrodites (ovarian and testicular tissue) were found to have a 46,XX karyotype. Four patients had an apparently 46,XYq− karyotype in which the normal Y chromosome was replaced by a small metacentric or submetacentric chromosome with no quinacrine-bright material. One fertile male with a Y chromosome lacking quinacrine-bright heterochromatin was ascertained through a population survey. A phenotypically normal 46,XX female with a familial translocation of quinacrine-bright Y heterochromatin to chromosome 15 was also studied.

DNA Extraction and Gel-Transfer Hybridization

DNA was prepared from peripheral leukocytes, cultured skin fibroblasts, or EBV-transformed lymphoblasts by published methods [21, 22]. Restriction digestion, electrophoresis, transfer, and hybridization of DNA were performed as described [23]. As specified below, each hybridization probe was used at either "reduced" or "high" stringency. "Reduced" stringency implies that hybridizations were carried out at 42°C and that the final wash was in 2 × SSC at 68°C or in 0.1 × SSC at 55°C. "High" stringency implies that hybridizations were carried out at 47°C and/or that the final wash was in 0.1 × SSC at 68°C.

DNA Hybridization Probes

With the exception of probes pDP34 and P1, all probes described below are plasmid subclones derived from a Y-enriched cosmid library [12, 24]. For many of the probes, we have indicated the names of the homologous DNA segments or loci (e.g., *DXYS5*, *DYZ1*) as assigned at the Human Gene Mapping Conference VII [25].

Probes 47a and 47z detect highly homologous sequences on the X and Y chromosomes (*DXYS5*) [9]. At high stringency, 47a detects a Y-specific *Taq*I fragment of 4.3 kb, while 47z detects a Y-specific *Taq*I fragment of 3 kb. These sites of X-Y homology are also detected by probe 47c [15]. 47a, 47c, and 47z are subclones from the same cosmid.

Probe 13d detects highly homologous sequences on the X and Y chromosomes (*DXYS7*) [9]. At high stringency, 13d detects a Y-specific *Taq*I fragment of 7 kb.

Probe 115 detects highly homologous sequences on the X and Y chromosomes (*DXYS8*) [9]. At high stringency, 115 detects a Y-specific *Taq*I fragment of 2.1 kb.

Probe 52d detects multiple loci on the Y chromosome as well as one on the X [12]. At reduced stringency, 52d detects Y-specific *Eco*RI fragments of 7 kb (restriction fragment 52d/A), 1.2 kb (52d/B), and 1.0 kb (52d/C; apparently an unresolved doublet—see text). The corresponding Y-specific *Taq*I fragments are 9 kb (52d/C; again, an unresolved doublet), 5 kb (52d/A), and 3 kb (52d/B).

Probe 50f2 [15] defines multiple Y-specific loci and an autosomal locus. At reduced stringency, 50f2 detects Y-specific *Eco*RI fragments of 10 kb (50f2/A), 7.5 kb (50f2/B), 6 kb (50f2/C), 4.5 kb (50f2/D), and 1.7 kb (50f2/E). The corresponding Y-specific *Taq*I fragments are 9 kb (50f2/E), 8 kb (50f2/D), 3.5 kb (50f2/A or 50f2/B), and 3 kb (an unresolved doublet, corresponding to 50f2/C and either 50f2/A or 50f2/B).

Probe 118 [15] detects numerous Y-specific restriction fragments. Four *Taq*I fragments whose presence or absence could be unambiguously determined were considered in this study: 7 kb (118/A), 6 kb (118/B), 5 kb (118/C), and 1 kb (118/D).

Probe pDP34 detects highly homologous sequences on the X and Y chromosomes (*DXYS1*) [5, 6]. At high stringency, pDP34 detects a Y-specific *Taq*I fragment of 15 kb.

Probe 64a7 [15] detects homologous sequences on the Y and on an autosome. At reduced stringency, 64a7 detects a Y-specific *Taq*I fragment of 4.5 kb.

Probe 12f detects sequences on autosomes and the X as well as on the Y [12]. At high stringency, 12f detects two or three Y-specific *Taq*I or *Eco*RI fragments. We scored for the presence or absence of an 8-kb, Y-specific *Taq*I fragment (corresponding to a 5-kb *Eco*RI fragment).

At reduced stringency, probe 49f [12] detects numerous Y-specific fragments. We scored only for the most intensely hybridizing Y-specific fragments (2.0- and 1.8-kb *Taq*I, or 2.8-kb *Eco*RI).

DYZ1 repeats were probed with a mixture of several cloned 3.4-kb *Hae*III repeated elements. *DYZ1* repeats have been detected at high stringency as a Y-specific *Eco*RI fragment of 3.4 kb or as several discrete Y-specific *Taq*I fragments of less than 0.6 kb.

DYZ2 repeats were examined using probe P1, a subfragment of the 2.1-kb *Hae*III repeated element [22, 26]. *DYZ2* repeats were characterized at high stringency as Y-specific smears of high molecular weight in both *Eco*RI and *Taq*I digests. In addition, discrete Y-specific fragments were also observed in *Taq*I digests.

RESULTS AND DISCUSSION

A Deletion Map of Y-specific DNA Sequences

DNAs from 19 XX males, two XX hermaphrodites, and six persons with cytogenetically detected abnormalities of the Y chromosome (table 1) were tested by hybridization for the presence of a number of Y-chromosomal sequences. Shown in figures 1 and 2 are autoradiograms from some representative hybridization experiments. DNAs were digested with the restriction en-

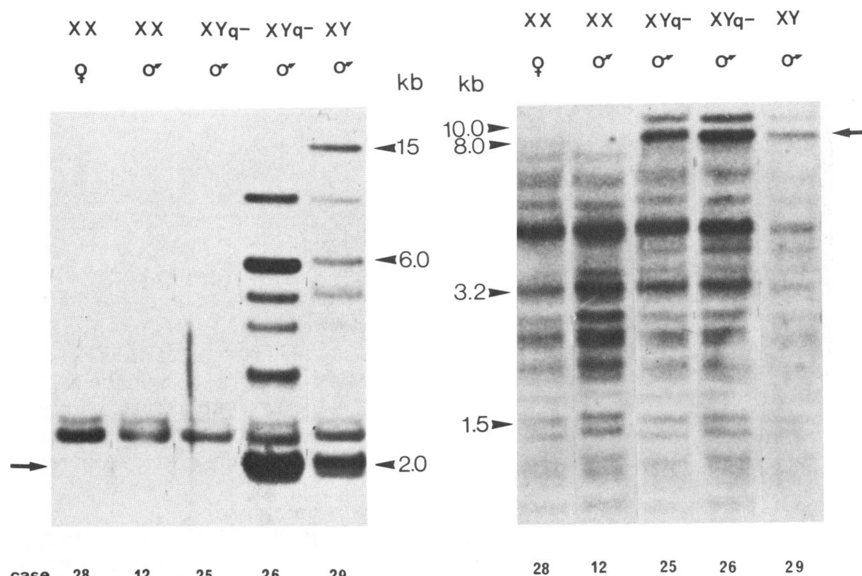

FIG. 1.—Hybridization of two probes detecting Yq-specific sequences to XX male and XYq− male DNAs. *Left panel*, ^{32}P-labeled probe 49f was hybridized to a gel transfer of *Taq*I-digested DNAs from a normal female, an XX male, two XYq− males, and a normal male. 49f detects two autosomal bands present in all of these individuals. 49f also detects a Y-specific 2.0-kb fragment in all normal males tested and in one of the two XYq− males shown here (case 26). 49f detects several additional Y-specific fragments; the size and presence of some of these is polymorphic (K. Y. Ngo, G. Vergnaud, C. Johnsson, G. Lucotte, J. Weissenbach, unpublished results, 1985), as can be seen in the comparison of XYq− male 26 with normal male 29. We have scored these and the other individuals in table 2 for the presence or absence of the nonpolymorphic Y-specific 2.0-kb fragment indicated by *the arrow*. *Right panel*, Probe 12f was hybridized to the same *Taq*I gel transfer. 12f detects many autosomal and X-chromosomal fragments [12] present in all of these individuals. In addition, 12f detects several Y-specific fragments; we scored for the presence or absence of the 8-kb fragment shown by *the arrow*.

donucleases *Taq*I or *Eco*RI and hybridized [27] with radiolabeled DNA probes detecting Y-specific restriction fragments. For each probe, we have scored for unambiguously Y-specific bands. The diversity of probe types is illustrated in figures 1 and 2. In figure 1 (left panel) is shown a probe that detects autosomal as well as multiple Y-specific bands, some of which are polymorphic. In figure 1 (right panel) is shown a probe detecting many autosomal and X-chromosomal bands; nevertheless, we were able to score unambiguously for a Y-specific 8-kb *Taq*I fragment. In figure 2 are shown two probes that each detect single-copy sequences on the X and Y chromosomes.

The results of these studies are summarized in table 2. The 27 individuals tested were scored for the presence or absence of 23 restriction fragments observed in normal males but not in normal females and therefore assumed to derive from the Y chromosome. Some probes (e.g., 50f2, 118, and 52d) detect multiple Y-specific fragments. For such probes, we scored individuals for the presence or absence of the particular Y-specific fragments indicated in table 2 (and described in MATERIALS AND METHODS). As indicated in table 2, we detected

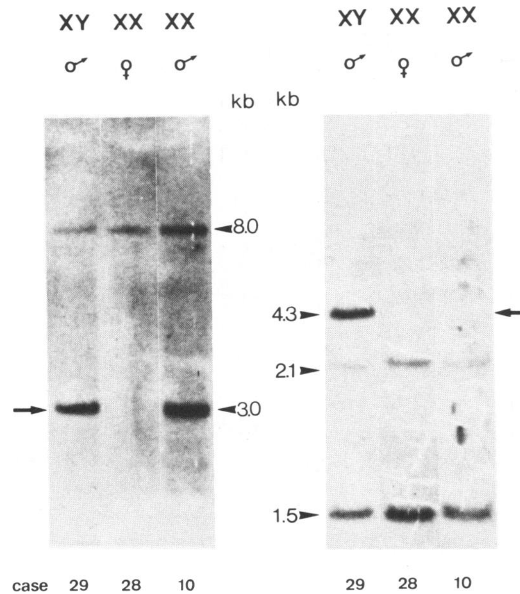

FIG. 2.—Hybridization of probes 47a and 47z to DNA of XX male 10. ^{32}P-labeled probes 47a (*left panel*) and 47z (*right panel*) were hybridized to gel transfers of *Taq*I-digested DNAs from a normal male, a normal female, and XX male 10. 47a detects an X-chromosomal fragment of 8 kb and a Y-specific fragment of 3 kb (*arrow*). 47z detects X-chromosomal fragments of 1.5 and 2.1 kb and a Y-specific fragment of 4.3 kb (*arrow*).

no Y-specific DNA sequences in nine individuals [two XX hermaphrodites, cases 1 and 2; and seven XX males, cases 3–9, whom we will refer to as Y(−) XX males]. In the other 18 individuals [cases 10–27, including 12 XX males, whom we will refer to as Y(+) XX males], we detected the presence of one or more Y-specific restriction fragments. In none of these 18 individuals did we detect all the Y sequences for which we tested; all of these sequences were present in each of the normal male controls (and, when tested, the fathers of the cases). Without exception, the *Taq*I or *Eco*RI restriction fragments detected in these 18 individuals (or, when tested, their fathers) were of the same size as those seen in normal males. We conclude that each of these 18 cases contains a portion but not all of the Y chromosome, and that, in principle, this might allow construction of a deletion map of the Y-specific DNA sequences.

Taken as a whole, the data in table 2 are consistent with the idea that, in each of these cases, only a single contiguous portion of the Y chromosome is present; that is, the Y-specific sequences can be ordered so that, in each of the patients tested, the Y sequences present are a single, uninterrupted cluster. In table 2, the Y-specific restriction fragments—the columns—have been so ordered. In each of the persons with cytogenetically detected abnormalities (cases 22–27), the chromosome-banding studies are consistent with the presence of a single, contiguous portion of the Y chromosome, but such studies are not sufficiently precise as to exclude more complicated rearrangements of the Y chromosome.

TABLE 2

Y-SPECIFIC RESTRICTION FRAGMENTS DETECTED IN XX MALES AND IN INDIVIDUALS WITH STRUCTURAL ABNORMALITIES OF THE Y CHROMOSOME

	CASE		Y-SPECIFIC RESTRICTION FRAGMENTS																	
			47a	47z	13d	115	52d/B	52d/C	50f2/A,B	118/A,B,C	pDP34	64a7	50f2/D	12f	52d/A	50f2/C,E	49f	118/D	*DYZ1**	*DYZ2**
Y(−) XX hermaphrodites	1	LGL208	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	CHM002	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
Y(−) XX males	3	CHM003	−	−	−	ND	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
	4	LGL200	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	5	LGL203	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	6		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
	7	PAR019	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
	8	PAR020	−	−	−	ND	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
	9	CHM006	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND
Y(+) XX males	10	CON101	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	11		+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	12	GRB027	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	13	GM2670	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	14	GM2626	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	15	LGL163	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	16	LGL197	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	17	CHM005	+	+	ND	+	ND	ND	+	+	−	−	−	−	ND	−	−	−	−	−
	18		+	+	+	+	+	r	+	+	−	−	−	−	−	−	−	−	−	−
	19		+	+	+	+	+	r	+	+	−	−	−	−	−	−	−	−	−	−
	20	LGL105	+	+	+	+	+	r	+	+	−	−	−	−	−	−	−	−	−	−
	21	LGL115	+	+	+	+	+	r	+	+	−	−	−	−	−	−	−	−	−	−
XYq− males	22	CHM004	+	ND	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	23	SL	+	ND	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	24	LL92	+	ND	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	25	CHM018	+	ND	ND	ND	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	26	CHM007	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
t(Y;15)female	27	CHM008	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Normal females	28		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Normal males	29		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

NOTE: DNAs from 27 individuals (karyotypes and phenotypes in table 1) were tested for the presence (+) or absence (−) of 23 Y-specific restriction fragments. (*) The other columns in the table refer to specific single or low-copy number restriction fragments detected by cloned probes (see MATERIALS AND METHODS). In the case of loci *DYZ1* and *DYZ2*, we are scoring for highly repeated, heterogeneous sequences. (r) In cases 18–21, bands 52d/C and 52d/B are of equal intensity, while in normal males and in cases 22–26, 52d/C is more intense than 52d/B; "r" indicates this relative reduction in intensity of 52d/C in cases 18–21. (ND) Not determined.

In this way, the patterns of Y-specific DNA sequences present in the 27 individuals tested allow us to generate a consistent deletion map of the human Y chromosome. In this map, each of the 23 Y-specific restriction fragments for which we tested is assigned to one of seven deletion intervals (fig. 3). These seven intervals can be ordered in a number of ways, all compatible with the presence of a single, contiguous portion of the Y chromosome in each of the individuals studied. Shown in figure 3*A* is one such arrangement of these seven intervals. This arrangement is consistent with each of the individuals studied having received a terminal, contiguous portion of the Y chromosome, that is, one which includes a telomere. An alternative arrangement of intervals is shown in figure 3*B*. This is only one of many possible models that presuppose that some of these individuals have received an internal, contiguous portion of the Y.

The present study extends the finding [15, 16] that many human XX males contain Y-specific DNA sequences but are heterogeneous with respect to the amount of Y-chromosomal material present. We detect Y-specific DNA sequences in the genomes of 12 of 19 XX males. In all cases, the Y-specific restriction fragments observed are of the same lengths as in normal males. The 12 Y(+) XX males can be divided into three classes according to the number of Y-specific fragments present. The DNA sequences present in these three classes comprise a nested series (table 2, fig. 3). The class 1 XX male carries only one Y-specific restriction fragment for which we tested (defining interval 1 in fig. 3). The class 2 XX males carry that same Y-specific fragment as well as three others (defining interval 2). The class 3 XX males carry all the Y-specific fragments present in class 2 as well as eight others (defining interval 3).

Orientation of the Deletion Map with Respect to the Arms and Centromere of the Y Chromosome

Infertility in males is sometimes associated with deletions of Yq, the long arm of the Y chromosome. Five males with microscopically visible deletions of the long arm of the Y (and an apparently intact short arm) and one female with a translocation of Yq heterochromatin to chromosome 15 were among those scored for the presence or absence of Y-specific restriction fragments. The sterile 46,XYq− males (cases 22–25) lack the *DYZ1* and *DYZ2* repeated sequences as well as certain single-copy Y sequences (table 2). These sterile 46,XYq− males can be divided into two classes according to the number of single-copy Y sequences they lack. Case 25 differs from cases 22–24 by the presence of the 8-kb *Taq*I fragment detected by probe 12f (defining interval 5 in fig. 3). A normal male with a nonfluorescent Y chromosome (case 26) lacks only the *DYZ1* and *DYZ2* repeats (defining interval 7) and differs from case 25 by the presence of several single-copy sequences (defining interval 6). Conversely, a 46,XX,der(15), t(Y;15) (q12;p11) female, with the quinacrine-bright distal portion of Yq translocated to chromosome 15, carries the *DYZ1* and *DYZ2* repeats (interval 7) but none of the Y-specific single-copy sequences listed in table 2 (intervals 1–6). However, we have detected another Y-specific single-copy sequence in this female (unpublished results).

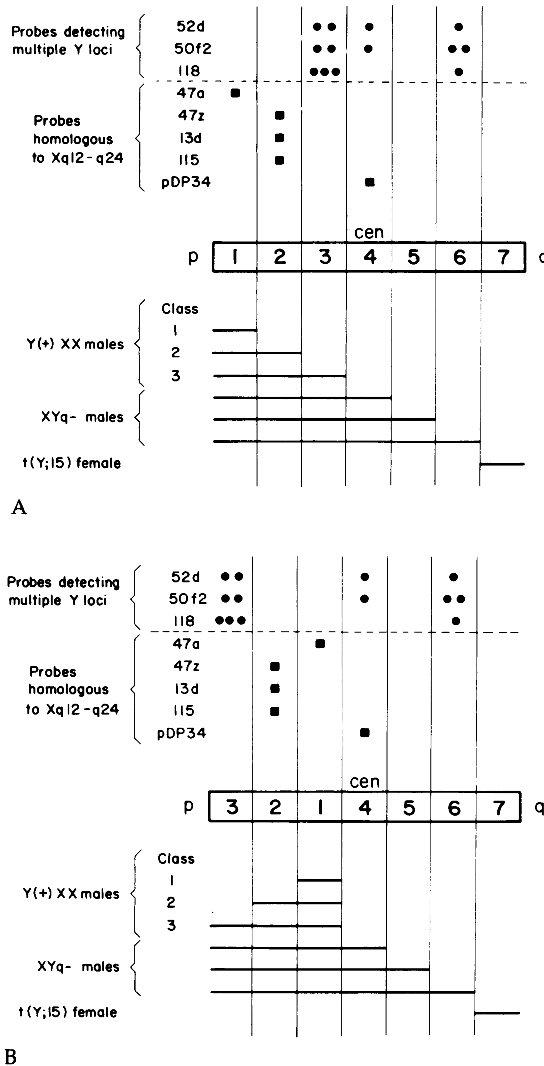

FIG. 3.—Seven intervals in a deletion map of the Y chromosome. *A*, An arrangement of the seven intervals consistent with the supposition that each of the individuals studied carries a terminal, contiguous portion of the Y chromosome. *B*, One of several possible arrangements of the seven intervals in keeping with the supposition that some of the individuals studied have received an internal, contiguous portion of the Y chromosome. In both (*A*) and (*B*), the seven intervals shown are defined by the various classes of Y(+) XX males, XYq− males, and the female with a Y;15 translocation. The Y-specific restriction fragments listed in table 2 can each be assigned to one interval according to their presence or absence in the genomes of these individuals. The short arm, centromere, and long arm of the Y chromosome are indicated by, respectively, "*p*," "*cen*," and "*q*." Probes 52d, 50f2, and 118 each detect multiple Y-specific restriction fragments (see text). *Solid circles* indicate the intervals to which those restriction fragments map. Probes 47a, 47z, 13d, 115, and pDP34 each detect highly homologous sequences on the human X chromosome in the region Xq12-q24. *Solid squares* indicate the intervals in which the Y-specific sequences they detect map.

These Yq deletions allow us to assign intervals 5, 6, and 7 to the long arm of the Y chromosome (fig. 3) and serve to orient the deletion map with respect to the long and short arms of the Y chromosome. Assuming that these Yq− chromosomes represent simple deletions (and not, for instance, translocation products), they all have the Y centromere. On the other hand, it seems unlikely that the Y(+) XX males, in whom no Y chromosomal material was detected cytogenetically, have the Y centromere. The Y centromere is found, then, in interval 4, which is present in all the XYq− males but absent in all the XX males (fig. 3). Interval 4 also contains the Y locus detected by probe pDP34 (*DXYS1*). This locus has been mapped to Yp by in situ hybridization [6]. Thus, interval 4 appears to include part of the short arm as well as the centromere of the Y chromosome. It follows that intervals 1–3 are entirely within Yp. These conclusions are based on the assumption that the rearrangement in the Yq− chromosomes is a simple deletion affecting Yq only. While the morphology of each Yq− chromosome is compatible with such a deletion, we cannot formally exclude more complicated rearrangements on the basis of cytogenetic studies. If events such as inversions or translocations were involved, the above conclusions might have to be modified.

The Y-specific DNA sequences that are present in Y(+) XX males are those in intervals 1–3, and we have assigned those intervals to Yp. All of the Y(+) XX males (and all of the XYq− males) have in common interval 1. Assuming that XX males have testes because of the presence of a male-determining portion of the Y chromosome, we can map that male determinant to interval 1. This assignment of the male determinant to Yp is in agreement with most karyotype-sex phenotype correlations [3]. Our findings argue strongly against the hypothesis [2, 28] that genes on both the short and long arms of the Y chromosome are required for testicular differentiation.

Our data are consistent with the hypothesis that XX maleness results from the translocation of a portion of Yp to another chromosome (possibly the X [14]) during or prior to meiosis in the father. One could envision the XX males having received a terminal contiguous portion of Yp, that is, a portion that includes the telomere. As stated earlier, the seven deletion intervals we have defined can be ordered in a number of alternative ways. Particularly in question is the ordering of intervals 1, 2, and 3 (for example, fig. 3*A* vs. fig. 3*B*). The model shown in figure 3*A* is consistent with the presence of a Y telomere in each of the individuals studied. According to this "terminal" model, the various classes of Y(+) XX males carry portions of Yp that include the telomere and extend a variable distance toward the centromere. However, it is conceivable that some if not all of the Y(+) XX males have received internal, contiguous portions of Yp. A number of arrangements of intervals 1, 2, and 3 are consistent with this "internal" model, and that shown in figure 3*B* is but one of these. We should be able to determine which is the correct model—terminal or internal—by studying additional anomalies of the Y chromosome with the DNA hybridization probes described here.

It should also be noted that, should an internal model be correct, some (but not all) of the Y sequences mapped to interval 4 could, in fact, define a new interval distal on Yp to intervals 1, 2, and 3. Such models cannot be excluded

by current data. Therefore, we define interval 4 as containing the centromere. As discussed below, the distribution of X-Y homologous sequences may shed some light on these issues.

Etiology of XX Maleness

The breakpoints on the Y chromosome vary among the Y(+) XX males. In all the Y(+) XX males we have studied, the breakpoint is apparently on Yp. In the case of XX male 10, the Y-specific sequence detected by probe 47a is present (left panel in fig. 2), while that detected by probe 47z is absent (right panel in fig. 2). As these two sequences occur only 35 kb apart on the normal Y chromosome (unpublished results), it appears that the Y breakpoint in XX male 10 falls within that 35-kb segment. Since no other Y DNA has been detected in this patient, it appears that, of Y sequences characterized to date, those homologous to probe 47a are among the closest to the male determinant(s).

Although the vast majority of XX males occur sporadically, a number of families with two or more XX males or hermaphrodites have been reported [13]. Affected individuals from two such families have been analyzed in this study. We have previously reported the presence of Y DNA in three related XX males (cases 13, 14, and 21 in table 2) from one of these kindreds [16]. The results suggested that, in that family, two XX males who are second cousins (cases 13 and 14) carry similar if not identical portions of the Y chromosome. In the present study, we have examined these two second cousins with six additional Y-DNA probes, and still we are unable to differentiate between the portions of the Y chromosome that they possess. As these second cousins are related through males only, their fathers carry genetically identical Y chromosomes. Perhaps a particular region of that Y chromosome is predisposed to breakage and translocation [16]. We have reported the absence of Y-specific sequences in the mothers of these related XX males [16]. We have also looked for but not found Y DNA in the mothers of eight of the sporadic XX males studied here (results not shown). Thus, it appears that XX males have received a male-determining portion of the Y chromosome from their fathers. In the second family studied, there are four siblings: two normal 46,XY males, one 46,XX true hermaphrodite (case 2), and one 46,XX male (case 3). As no Y DNA was detected in either case 2 or case 3, the occurrence of testes in these two related 46,XX individuals is as yet unexplained.

It seems likely that at least some of the XX males in whom no Y DNA has been detected may contain portions of Yp smaller than those found in the Y(+) XX males. Phenotypically, these Y(−) XX males, in whom none of the presently available probes detects Y DNA, do not differ consistently from the Y(+) XX males. In fact, there is little phenotypic variation among XX males, and we have found no phenotypic character that correlates with the variable amount of Y DNA they carry.

Structural Organization of the Human Y Chromosome

These studies provide considerable information as to the organization of DNA sequences within the Y chromosome. Under the hybridization conditions used, probes 50f2, 52d, and 118 detect multiple Y-specific restriction frag-

ments. The fragments detected by each of those probes are not all clustered within single intervals. We noted that in class 3 XX males bands 52d/C and 52d/B are of equal intensity, while in normal males (or in the XYq− males), 52d/C is more intense than 52d/B (table 2). We conclude that band 52d/C is at least a doublet, composed of one copy found in interval 3 and one or more copies in interval 4. Probe 118 detects many Y-specific *Taq*I fragments; of the four Y-specific fragments that could be scored unambiguously, three (118/A,B,C) map to interval 3, while one (118/D) maps to interval 6 (fig. 3). Thus, probes 50f2 and 52d each detect sequences in intervals 3 (on Yp), 4, and 6 (on Yq), and probe 118 detects sequences in intervals 3 and 6 (and probably elsewhere). These Yp-Yq homologies may be the result of one or more duplications of portions of the Y chromosome during evolution. Families of highly homologous DNA sequences have also been observed on the mouse Y chromosome [29, 30].

Numerous Y DNA sequences are highly homologous to sequences located between bands q12 and q24 of the X chromosome [6, 9]. As shown in figure 3, many of these Xq-homologous sequences are clustered in intervals 1 and 2 (47a and 47z [locus *DXYS5*], 13d [*DXYS7*], 115 [*DXYS8*]; and unpublished results). In contrast, *DXYS1*, the Xq-homologous locus detected by probe pDP34 maps to deletion interval 4. Apparently most if not all of these sequences highly homologous to Xq12-q24 are found on Yp. (*DXYS1* has been mapped to Yp by in situ hybridization [6].) *DXYS1* is found on the X but not on the Y chromosome in hominoid apes and thus appears to have been transposed from the X to the Y chromosome since the divergence of human from chimpanzee [6]. Like *DXYS1*, at least some of these other X-Y loci are X-limited in chimpanzee (unpublished results). At least some of these other Xq-homologous loci [9] also exhibit a degree of X-Y sequence homology comparable to the value of 99% observed at *DXYS1* ([6] and our unpublished results, 1985). Thus, these preliminary studies suggest that many of these X-Y homologies are the result of transposition from Xq to Yp during recent evolution. As discussed above, there is some uncertainty as to the ordering of intervals 1, 2, and 3, which we have assigned to Yp. According to the previously discussed "terminal" model (fig. 3*A*), highly X-homologous sequences are located in two distinct areas on Yp: pDP34 is near the centromere, in interval 4, while a group of other X-homologous sequences (probes 47a, 47z, 13d, and 115) are found more distally, in intervals 1 and 2. If this order is correct, then either multiple Xq-Yp transpositions occurred during recent evolution or, following a single transposition, some event resulted in the intercalation of Y-specific DNA (in interval 3) among the X-Y sequences. However, according to the "internal" model, intervals 1, 2, and 3 would be reordered. In particular, the arrangement shown in figure 3*B* would be consistent with the relative proximity on Yp of all the sequences highly homologous to Xq12-q24.

From the results thus far presented, it is clear that there are numerous lengthy Y-specific sequences in different deletion intervals on Yp. This observation is inconsistent with the idea that Xp and Yp are homologous along the entire length of Yp. Therefore, Xp-Yp synapsis in male meiosis [31–33] cannot reflect continuous DNA sequence homology to Xp along the entire length of Yp.

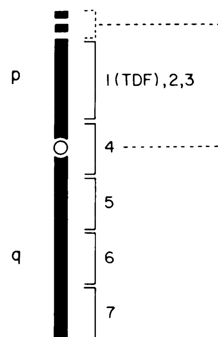

FIG. 4.—Cartoon of the genetic deletion map of the human Y chromosome. *The heavy vertical line* represents Yp and Yq, although no implication about cytological distances is intended. *The open circle* indicates the centromere. Intervals 1, 2, and 3 may or may not include the Yp telomere. The testis-determining gene(s) or factor(s) (TDF) lie in interval 1. Interval 4 includes the centromere, but individual sequences within interval 4 may, in fact, lie on distal Yp as shown by *the dotted bracket*.

The 2.1- and 3.4-kb *Hae*III repeats (*DYZ2* and *DYZ1*, respectively) are clustered in deletion interval 7 (fig. 3), which corresponds to the quinacrine-bright, heterochromatic, distal part of Yq. This result is in agreement with numerous earlier observations [21, 34–38]. However, the presence of small numbers of *DYZ1* and/or *DYZ2* repeats in other regions of the Y chromosome cannot be excluded. Conversely, the heterochromatic portion of Yq may not be composed exclusively of these repeats; we have evidence of a single-copy sequence in interval 7 (unpublished results), and there are reports of other single-copy sequences on distal Yq [7, 39].

The testis determinant(s) is not the only gene on the Y chromosome. However, genetic analysis of the mammalian Y chromosome has long been impeded by its haploid state. Unlike the other nuclear chromosomes, the Y has little opportunity to recombine with a homolog, making genetic linkage studies of the Y chromosome difficult if not impossible. This at least partially accounts for the relative dearth of genes mapped to the Y chromosome in the mouse [40] or human [41]. Historically, attempts to establish the Y-linkage of certain traits have been inconclusive because of the difficulty of distinguishing true Y-linked inheritance from sex-limited expression [42]. Nonetheless, there is strong evidence for a number of genes on the Y chromosome in addition to the male determinant(s). A structural gene for the antigen 12E7 has been mapped to the human Y chromosome [43]. A structural or regulatory locus for the H-Y antigen probably maps to the Y chromosome in both the mouse and human; however, H-Y transplantation antigen is likely not directly involved in testis determination [44]. Two genes have been assigned to the weakly fluorescent proximal portion of Yq (band Yq11): a gene affecting spermatogenesis [45] and a gene affecting height and tooth size [46]. It may soon be possible to correlate the loss of specific parts of Yq11, as determined using the probes we describe here, with effects on these and other phenotypes. Thus, we can anticipate the assignment of phenotypically significant Y DNA sequences to intervals on the DNA-based deletion map we have described.

CONCLUSION

Shown in figure 4 is a cartoon of the genetic deletion map of the human Y chromosome as we now envision it. Deletion intervals 1, 2, and 3 are clearly within Yp but cannot be definitively ordered with respect to each other. Interval 1 contains the testis-determining gene(s) as defined by the Y(+) XX males. Interval 4 contains the centromere, although some probes now classed in interval 4 could, in principle, be located distal to intervals 1, 2, and 3 on Yp. Intervals 5, 6, and 7 are on Yq in the order shown. Our methods provide information as to the order of but not the distance between DNA sequences on the Y. Exact correlation of this genetic deletion map with the cytogenetic map of the Y awaits further study.

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