hours after injection of 1*M* NaCl (1 percent of body weight), in the absence of water, were 6.11 ml and 312.0 meq/liter in rats with lesions and 9.5 ml and 336.6 meq/liter in controls. At the end of the 5-hour combined-stimulus test, values in blood were as follows: sodium, 139.3 meq/liter in rats with lesions and 141.3 meq/liter in controls; hematocrit, 43.6 in animals with lesions and 42.8 in controls; osmolarity in plasma, 303.5 milliosmols in animals with lesions and 304.6 milliosmols in controls; and protein in plasma, 6.4 g/100 ml in animals with lesions and 6.7 g/100 ml in controls.

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Human Y-Chromosome-Specific Reiterated DNA

Abstract. *Radiolabeled reiterated DNA specific for the human Y chromosome has been obtained by extensive reassociations between [³H]DNA prepared from men and excess DNA from women. These highly purified labeled sequences reassociate only with DNA from individuals with a Y chromosome. The percentage of Y-chromosome-specific DNA isolated from individuals with differing numbers of Y chromosomes is a function of the number of Y chromosomes present. The purified Y-chromosome-specific sequences may represent between 7 and 11 percent of the human Y chromosome.*

The mechanisms by which the Y chromosome engenders maleness are unknown. Investigation has been handicapped by the lack of Y-chromosome-specific gene assignments. In this report we describe the isolation of ³H-labeled reiterated DNA unique to the human Y chromosome.

Whole blood lymphocytes obtained from individuals bearing the chromosome constitution 46,XX; 46,XY; 47,XYY; or 48,XYYY were labeled continuously in cultures containing [³H]thymidine (*1*). DNA isolated (*2*) from each of these cultures was sonicated (*3*), and reiterated sequences were enriched by reassociation to C_0t value of 46 (*4*). The reassociated reiterated sequences, representing 30 to 40 percent of the genome, were collected on hydroxylapatite and subsequently fractionated by stepwise elution with increasing concentrations of phosphate buffer, *p*H 6.8 (*5*). Preliminary experiments indicated that the most stable duplexes, that is, those eluted by phosphate concentrations between 0.25 and 0.3*M*, were slightly enriched for DNA sequences of the Y chromosome. These stable duplexes were mixed with a 5000-fold excess of unlabeled DNA from a 46,XX woman. The mixture was incubated to C_0t 460. Those sequences failing to reassociate and therefore not binding to hydroxylapatite were collected and assayed (*6*) for Y-chromosome-specific sequences. For each individual, the percentage of ³H-labeled Y-chromosome-specific sequences present in the entire genome was calculated as the arithmetic product of the amount of ³H-labeled DNA recovered after each step, and the percent of the final fraction assayed (*6*) as Y-chromosome-specific (Table 1). As seen, this percentage is a linear function of the number of Y chromosomes present in each subject. Despite low recovery of stable duplexes from the DNA of the 46,XY subject and consequently a poor fit to linearity in this instance, the overall correlation coefficient of the regression of the percentage of Y-chromosome-specific DNA on Y-chromosome dose is .99.

These results (Table 1) establish that Y-chromosome-specific sequences indeed exist. Their further purification was facilitated by an increase in labeling over that attainable in lymphocyte culture. This was achieved by using the nick translation function (*7*) of DNA polymerase I (*Micrococcus luteus*) (Miles Laboratories, Inc.) to incorporate [³H]TTP (thymidine triphosphate) (New England Nuclear, 40 to 60 c/mmole) into isolated DNA from a 47,XYY individual. Prior to labeling, reiterated DNA from this subject was prepared by reassociation to C_0t equal to 46. Three percent of the genome was isolated as stable duplexes by elution with high salt from hydroxylapatite and then nick-translated. The specific activity of products ranged from 5×10^7 to 1×10^8 dpm/μg. The labeled product was reassociated to a whole genome C_0t of 46. Fifty percent of this product formed duplexes, which were collected on hydroxylapatite. The eluted duplexes were subsequently reassociated with a 40,000-fold excess of unlabeled DNA from a 46,XX subject. Those sequences which failed to reassociate, that is, 20 percent of material entering this step, were exposed to a second challenge with excess DNA from a 46,XX individual. ³H-Labeled sequences which again failed to reassociate (73 percent of input) were assayed (*6*) for Y-chromosome specificity. The portion of reiterated labeled DNA that remained reassociable was now completely specific for the Y chromosome (Fig.

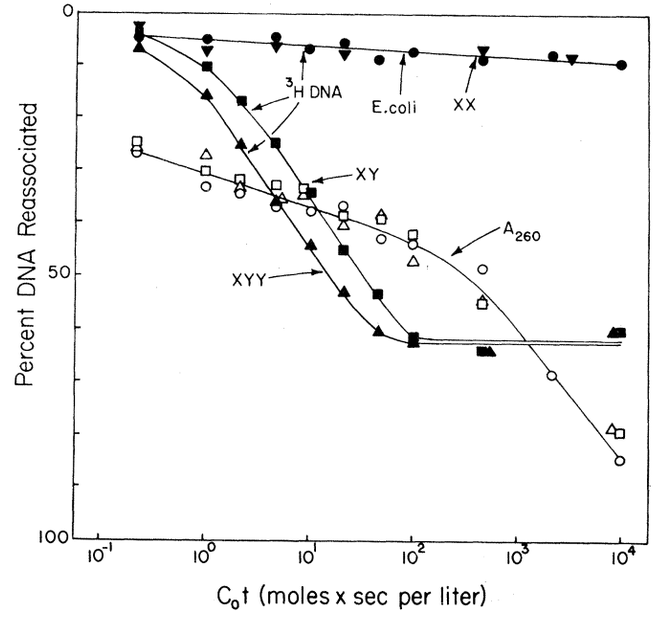

Fig. 1. Reassociation curves between a fraction of ³H-labeled DNA from a 47,XYY individual and excess whole genome DNA from various sources. The fraction of DNA from the XYY individual represents the stable duplexes isolated from hydroxylapatite after reassociation to C_0t 46 (*4*), thereafter labeled with [³H]TTP by nick translation (*7*), and then twice challenged with excess whole genome DNA from a 46,XX individual. The ³H-labeled sequences that twice failed to reassociate when challenged were then reassociated, as shown here, with more than a 10,000-fold excess of whole genome DNA from 46,XY ■—■, 47,XYY ▲—▲, 46,XX ●—● individuals, and *Escherichia coli* ▼—▼. Reassociation of labeled sequences was analyzed radiometrically. Reassociation of the unlabeled whole genome human DNA, monitored optically at A_{260}, is indicated by the open symbols: 46,XY □—□, 47,XYY △—△, and 46,XX ○—○. The analyses of *E. coli* DNA at A_{260} are not shown; it reassociated to approximately 90 percent with a $C_0t_{1/2}$ of 4. Reassociation conditions were the same as described in (*6*).

Table 1. The percentage of Y-chromosome-specific DNA isolated from individuals with differing numbers of Y chromosomes. Labeled DNA was prepared from each individual by the addition of [^{3}H]thymidine (New England Nuclear, 40 to 60 c/mmole, 100 μc per 10 ml of culture) to short-term lymphocyte cultures containing 0.5 ml of whole blood stimulated by phytohemagglutinin (*1*). After 4 days of culture at 37°C, cells were harvested. The specific activity of the DNA isolated from the nuclei (*2*) ranged between 2×10^5 and 4×10^5 dpm/μg. Yields for each subject were 100 to 150 μg of DNA per ten cultures. The DNA was sonicated and reassociated in the presence of *E. coli* DNA as carrier. Thereafter, labeled Y-chromosome-specific reiterated DNA sequences from each individual were enriched from stable duplexes by a single challenge with an excess of whole genome DNA from a normal woman. The enriched labeled sequences were then assayed (*6*) for Y-chromosome specificity.

Y chromosomes present* (No.)	^{3}H-labeled DNA			
	Percent forming stable duplexes at C_0t 46	Percent stable duplexes not reassociated with excess 46,XX DNA	Percent DNA from column 3 assayed (*6*) as Y-chromosome-specific	Calculated percent Y-chromosome-specific DNA in genome†
0	4.5	15	0‡	0§
0	4.5	12	0‡	0§
1	1.3	23	3	.009 ‖
2	3.7	14	7	.036
3	3.6	10	16	.058§
3	3.6	9	18	.058§

*Determined by karyotype analysis of each individual's cultured cells. †Each value was calculated as the product of three terms: (percent dpm forming stable duplexes, second column); (percent of stable duplexes failing to reassociate to DNA from a normal woman, third column); and (the percent of DNA in the third column assayed (*6*) as Y-chromosome-specific, fourth column). The calculation for an individual with three Y chromosomes (bottom row) is illustrated as follows: (3.6 percent) (9.0 percent) (18 percent) = 0.058 percent. ‡Values for the 46,XX female, when twice assayed (*6*) for Y-chromosome specificity, were within the error of the assay. §Two independent determinations were used to calculate the percent Y-chromosome-specific DNA in the genome. ‖The calculated low value is discussed in the text.

1). While 60 percent of the labeled 47,XYY-derived DNA which survived purification could reassociate with whole genome DNA from a 46,XY individual, its reassociation with whole genome DNA from a 46,XX individual was indistinguishable from that seen with *Escherichia coli* DNA. Equivalent results have been obtained in individual reassociation assays between these highly purified labeled Y-chromosome-specific sequences and excess DNA isolated from seven other males and five females. Further verification of Y-chromosome specificity arises from our finding (Fig. 1) that the rate of reassociation between the highly purified ^{3}H-labeled sequences and whole genome DNA from a 47,XYY individual is twice that of its reassociation rate with whole genome DNA from a 46,XY individual.

Calculations based on the same logic as outlined in Table 1 for [^{3}H]DNA from lymphocytes indicate that Y-chromosome-specific sequences prepared, on two separate occasions, from nick-translated DNA represent approximately 0.1 percent of the genome. Since the Y chromosome represents 1 to 1.5 percent of the genome (*8*), these sequences account for 7 to 11 percent of the Y chromosome. These percentages of the Y chromosome are considerably greater than the corresponding estimates of $\sim$1.2 to 1.8 percent obtained from DNA labeled during lymphocyte culture (Table 1). The difference between the two estimates may reflect (i) the influence of differing labeled DNA piece size (*3*) on recovery and assay or (ii) differences in the specific activity of Y-chromosome-specific sequences relative to the rest of the genome. Whatever the explanation, we emphasize that each kind of estimate is reproducible and the discrepancy between them does not detract from the demonstration that Y-chromosome-specific tracts exist (Table 1 and Fig. 1) and can be isolated (Fig. 1).

The relation of these Y-chromosome-specific DNA sequences to male differentiation is unclear. It may be revealed by correlating the presence of these sequences with the various phenotypes associated with particular qualitative abnormalities of the Y chromosome.

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References and Notes

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3. DNA was sonicated (30 watts for 5 minutes) at a concentration of 100 to 500 μg/ml in standard saline citrate (*2*) with a Branson model W185 Sonifier (Heat Systems–Ultrasonics, Inc.) fitted with a microtip. DNA labeled in cell culture and all driver DNA was approximately 500 nucleotides in size when analyzed in alkaline sucrose gradients [F. W. Studier, *J. Mol. Biol.* **11**, 373 (1965)]. After nick translation, labeled DNA was between 75 and 100 nucleotides in length.
4. C_0t is the product of the concentration of DNA in moles per liter times the time of incubation in seconds.
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6. The assay was performed as follows: The percent Y-chromosome-specific DNA was estimated by comparing (i) reassociation between labeled DNA and excess DNA isolated from a 46,XY male; and (ii) reassociation between the same labeled DNA and excess DNA from a 46,XX female. The difference [(i) minus (ii)] in the reassociation of the labeled DNA with these two unlabeled sources represents the percentage of the labeled DNA which is Y-chromosome-specific. Prior to assay, samples were boiled for 5 minutes in 0.12*M* phosphate buffer (*p*H 6.8) and then immediately incubated at 60°C. One-milliliter hydroxylapatite beds at 60°C were used to fractionate 5 A_{260} units of DNA. Reassociated duplexes were eluted with 0.3*M* phosphate buffer.
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9. We thank D. S. Borgaonkar for assistance and R. Stern for criticism. Supported by NIH grants 5P01-GM-19489 and 5R01-HL-15026.

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