the survival of haploid spores resulting from meiosis of diploids containing pRB111 integrated at actin. If such a null mutation is lethal, the spores that inherit the disrupted gene (and therefore the ura3$^+$ allele carried on the plasmid) should fail to grow, while the spores that inherit the intact actin gene should grow and show a ura$^-$ phenotype. The diploids carrying the plasmid integrated into the *ura3* gene serve as controls; they should all be viable and the ura$^+$ phenotype should segregate 2:2.

The results of tetrad analysis are shown in Table 1. Transformants 3 and 4 (pRB111 integrated into the *ura3* gene) gave mainly four viable spores per ascus, with no tetrad having fewer than three. The ura$^+$ phenotype segregated 2:2 and showed normal linkage to the *can1* locus, which resides on the same chromosome arm (linkage data not shown). Transformants 1, 2, 5, and 6 (all expected to have pRB111 integrated into the actin gene on the basis of the gel-transfer results) showed the pattern of viability expected for segregation of a recessive lethal mutation. Most importantly, none of the 100 spores were ura$^+$, indicating very tight linkage of the lethal mutation to the integrated plasmid. The one exceptional tetrad with three viable spores was tested further and apparently represents a gene conversion event that resulted in removal of the lethal mutation as well as the ura3$^+$ marker.

Our results indicate that disruption of the actin gene results in a recessive lethal mutation and justifies the conclusion that the actin gene encodes a function essential for germination of haploid spores. It seems very probable that the actin gene is also essential for vegetative growth of yeast cells, a conclusion supported by the construction (*12*) of three temperature-sensitive mutant alleles of the actin gene.

Since tetrad analysis is carried out by micromanipulation, the fate of individual spores that have inherited the disrupted actin gene could easily be followed by microscopy. Of 30 spores that failed to form microcolonies after 24 hours on a rich medium, 28 failed to bud at all and 2 possessed tiny buds. This result suggests a specific defect in bud emergence or in the initiation of a cycle of cell division.

The technique of gene disruption described above should provide a simple, definitive, and general method for inducing a null mutation in any yeast gene which has been cloned. In experiments very similar to those described above, it has been possible to demonstrate that the single yeast β-tubulin gene encodes an essential function (*13*). Gene disruption results in an insertion mutation which is marked by the acquisition of a selectable character carried by the integrating plasmid. Null mutations of this type, therefore, can be stably maintained by selection in diploids and reliably scored in tetrad analysis. As a genetic tool, use of gene disruption mutants has many of the advantages characteristic of transposon insertion in prokaryotes (*14*), including complete loss of function, indissolubly linked selectable marker, and acquisition of additional DNA at the position of the mutation.

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Genetic Length of a Human Chromosomal Segment Measured by Recombination Between Two Fragile Sites

Abstract. *Two families were studied in which the same homolog of chromosome pair 10 expressed both the fragile sites on the long (q) arm at 10q23 and 10q25. Recombination between the fragile sites was observed in 3 of the 27 offspring in whom it could occur. The genetic length of chromosome between the fragile sites was 11 female centimorgans within a 95 percent probability interval of 4 to 28 centimorgans. This estimate of genetic length is comparable to those obtained with other methods.*

Fragile sites are morphological features of human chromosomes that can be recognized cytologically when lymphocytes are cultured under appropriate conditions (*1*). Since any fragile site is always expressed at the same locus in individuals from the same kindred, kindreds in which fragile sites are segregating can be used for gene mapping (*2*). Two fragile sites have been described on chromosome 10. One, in band 10q23, is expressed only under conditions of folic acid and thymidine deprivation (*1*), and the other, in band 10q25, is expressed only if bromodeoxyuridine or bromodeoxycytidine is present in the tissue culture medium for some hours before harvest (*3*). The discovery of two families in which both of these fragile sites were present on one chromosome (Fig. 1) provided an opportunity to measure the genetic length of the segment between the fragile sites by segregation analysis.

The two families are not known to be related to each other, although both are of British origin. The families were ascertained through mildly retarded girls, but other members of these families who have the fragile sites on chromosome 10 were normal. Examples of number 10 chromosomes expressing both the fragile sites are shown in Fig. 2. On G-banded preparations, one fragile site is at the distal end of 10q23 (*1*), probably at 10q23.32 or 10q23.33, and the other in the middle of 10q25 (*3*), probably at 10q25.2 (*4*).

The frequency of expression of the fragile sites is shown in Table 1. One member of family Ho, III-12, has been omitted from consideration because in the small number of cells available for examination, it was not possible to be sure that fra(10)(q25) was absent. Fragile sites were present on the same homolog of chromosome 10 in several individuals, including I-6 from family Ho and I-1

from family Ch. In those individuals in whom the fragile sites were not found, at least 50 metaphases were usually examined for each fragile site.

Three recombinants were detected in family Ho, but none were seen in family Ch. Recombination occurred in 3 of the 27 offspring of females who expressed the fragile sites on the same homolog of chromosome 10. The recombination fraction is 11 percent (3/27), and since double crossovers are unlikely for such a small segment, the estimate of the genetic length of chromosome between the fragile sites in females is 11 centimorgans (cM) with a 95 percent probability interval of 4 to 28 cM (*5*). In general, the female map length is approximately twice that of males (*6*). On this basis the length of segment between the fragile sites in males would be about 6 cM with 95 percent probability interval of 2 to 21 cM (*5*), provided the general relationship between male and female recombination rates is applicable to this chromosomal segment.

An estimate of the genetic length of the segment of chromosome between the fragile sites from meiotic studies in males is 3.8 cM, with a possible maximum length of 13.5 cM, according to Hultén's model (*7*). Direct measurement of the segment between the bands expressing the fragile sites from prometaphase chromosomes (*4*) shows that the segment is about 18 percent of mitotic length of the long arms of chromosome 10 and that the approximate position of the segment is the proximal half of the distal third of the long arm of the mitotic chromosome. The meiotic map of the human genome put forward by Cook *et al.* (*6*) indicates that the distal third (in mitotic length) of the long arm of chromosome 10 is approximately 36 cM for males. The segment between the fragile sites corresponds to a male length of approximately 19 cM with Cook's map.

Table 1. Frequency of expression of the fragile sites in family members.

Family member	10q23	10q25
	Family Ch	
I-1	25/50	15/50
II-1	14/50	13/67
II-3	14/50	5/40
	Family Ho	
I-6	4/20	4/20
II-4	11/58	14/45
II-8	0/50	7/73
II-11	6/20	4/20
II-13	9/40	3/20
II-16	6/50	5/40
II-18	12/20	5/50
III-2	16/50	19/25
III-4	27/90	8/44
III-7	7/20	6/20
III-9	3/38	7/20
III-11	9/30	0/50
III-12	0/50	0/8
IV-1	7/25	0/65

There is good agreement between the estimates of the genetic length of the chromosomal segment between the fragile sites from segregation analysis and meiotic observation. Furthermore, the 95 percent probability interval of the estimate from segregation analysis encompasses the estimate from Cook's meiotic map, but Hultén's maximum estimate is shorter than that obtained from Cook's map. Cook *et al.* (*6*) have indicated they believe that Hultén's model underestimates genetic length.

The possibility that fragile sites could affect crossing-over should be considered. Crossing-over occurs in chromosomes in which the DNA is compacted, although not to the degree seen in C-metaphases. Fragile sites are defects in chromosome compaction for mitosis. If this defect in compaction also holds for meiosis, the fragile sites could in some way inhibit or possibly even facilitate recombination. This could lead to biased estimates of genetic length when fragile sites are used as genetic markers for linkage studies. The level of recombination observed between the fragile site at 16q22 and the haptoglobin locus *Hp* (*2*) may help to clarify this issue when *Hp* is precisely localized by methods other than linkage with the fragile site.

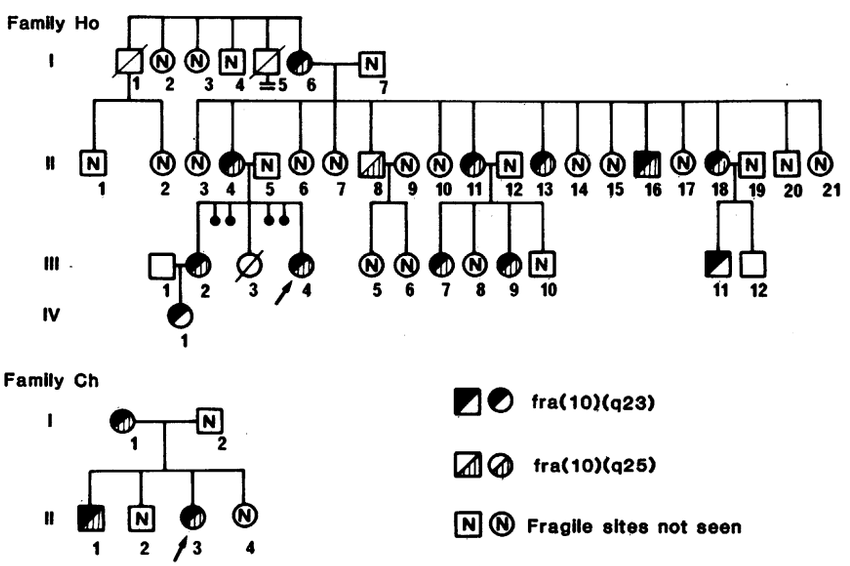

Fig. 1. Pedigrees of the two families.

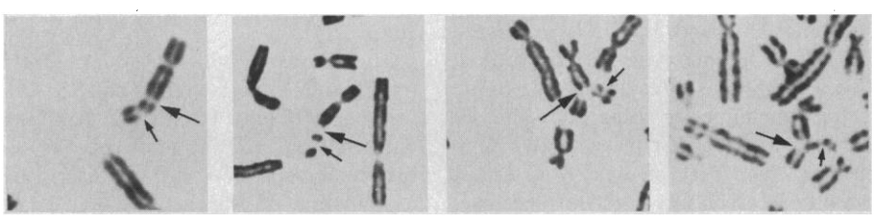

Fig. 2. Chromosome 10 from four metaphases showing both fragile sites expressed. The fragile site at 10q23 is indicated by the broad arrow and the one at 10q25 by the small arrow. To induce simultaneous expression of both fragile sites, lymphocytes were cultured in MEM-FA (*1*), and bromodeoxycytidine (75 mg/liter) was added 6 hours before harvest.

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