independent positive clones were sequenced and found to be identical to the normal OTC sequence with the exception of a single base change. This C to A transversion, which was found 348 bp downstream from the translation initiation point within two nucleotides of the position estimated from the RNase A cleavage pattern, results in the replacement of a histidine residue with an asparagine residue at amino acid position 117.

To ensure that the mutation that we had identified was responsible for the *spf* phenotype, we examined the expression of the wild-type OTC cDNA and an OTC cDNA bearing the C to A transversion in a transient assay by means of the eukaryotic expression vector p91023(B) and COS cells (*14*). The mutant cDNA generated an OTC with the same characteristic increase in activity at elevated *p*H that had been reported elsewhere for OTC extracted from *spf* mouse livers. In contrast, the wild-type clone gave an OTC with slightly reduced activity at high *p*H (Table 1) (*4*). Thus, it is almost certain that the DNA sequence change we have identified is the bona fide *spf* mutation, and not a DNA sequence polymorphism that is unrelated to the functional defect in the *spf* OTC gene.

The characterization of the *spf* mouse mutation at the nucleotide level improves the utility of this model for human OTC deficiency and provides the basis for simple methods to detect the *spf* mutation in mouse strains. Additionally, the strategy of mutation localization by RNase A cleavage followed by PCR amplification for rapid molecular cloning and sequencing is now shown to be a practical and simple method for the analysis of a new mutation.

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13. Amplification by polymerase chain reaction: First strand cDNA was synthesized from 5 μg of poly(A)$^+$ *spf* mouse liver RNA (*9*). PCR amplification was carried out with 100 ng of the cDNA and 1 μ*M* of each primer in 100 μl of a final reaction mixture containing 10 m*M* tris, *p*H 7.5, 10 m*M* $MgCl_2$, 5 m*M* dithiothreitol, and 1 m*M* each of dATP, dCTP, dGTP, and dTTP. After heating for 3 minutes at 95°C, the sample was cooled to 30°C to allow the primers to anneal; 5 units of DNA polymerase I (Klenow fragment) was added, and the mixture was incubated for an additional 3 minutes. This cycle was repeated ten more times. DNA was extracted with phenol, partially purified through a Sephadex G50 "minicolumn", digested with Bam HI and Xho I, then ligated directly into the Bam HI and Sal I sites of the M13 vector, mp9.
14. A full-length OTC cDNA bearing the *spf* mutation was constructed from the PCR-amplified material and wild-type OTC cDNA, then subcloned into p91023(B) [G. G. Wong *et al.*, *Science* **228**, 810 (1985)] and introduced into COS cells [Y. Gluzman, *Cell* **23**, 175 (1981)] by the DEAE-Dextran transfection method [H. Luthman and G. Magnusson, *Nucleic Acids Res.* **11**, 1295 (1983)]. Cell extracts were prepared after 48 hours and OTC activities were determined by a colorimetric assay (*4*). A β-galactosidase expression construct (pRSVZ) [G. R. MacGregor, A. E. Mogg, J. F. Bourke, C. T. Caskey, *Somat. Cell Molec. Genet.* **13**, 253 (1987)] was cotransfected with the OTC plasmids and the OTC activities from duplicate experiments were normalized to the relative β-galactosidase activities. Control transfections with pRSVZ alone yielded negligible OTC activity.
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23 December 1986; accepted 27 May 1987

Physiological Evidence for Serial Processing in Somatosensory Cortex

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Removal of the representation of a specific body part in the postcentral cortex of the macaque resulted in the somatic deactivation of the corresponding body part in the second somatosensory area. In contrast, removal of the entire second somatosensory area had no grossly detectable effect on the somatic responsivity of neurons in the postcentral cortex. This direct electrophysiological evidence for serial cortical processing in somesthesia is similar to that found earlier for vision and, taken together with recent anatomical evidence, suggests that there is a common cortical plan for the processing of sensory information in the various sensory modalities.

THE SECOND SOMATOSENSORY AREA (SII), like the postcentral somatosensory strip, has long been thought to receive a major projection from the ventroposterior nucleus (VP) (*1*), the principal somatic relay nucleus of the thalamus. It has therefore been assumed that the processing of tactile information proceeds in parallel in these two cortical regions. Recently, however, this assumption of parallel processing in SII and the postcentral cortex has been brought into question by anatomical evidence suggesting that VP provides only a sparse input to SII (*2*). A possible alternative source of somatic activation of SII neurons is the postcentral somatosensory cortex, because each of the cytoarchitectonic fields (areas 3a, 3b, 1, and 2) of which this cortex is comprised has been found to project densely on layers IV and lower III of SII (*3*). The electrophysiological results we report not only support this alternative possibility but also, by showing that postcentral ablations render SII somatically unresponsive, demonstrate that the input from the postcentral cortex is essential for the somatic activation of SII. Our results suggest that an important aspect of sensory processing in touch is carried out sequentially in the cortex, by transmission of information from lower order to higher order stations, in an arrangement similar to that for sensory processing in vision.

Single- and multi-unit activity was recorded in 11 hemispheres of seven macaques (five *Macaca mulatta* and two *Macaca fascicularis*) anesthetized with a mixture of halo-

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thane and nitrous oxide. Electrode penetrations were placed 0.5 to 1.0 mm apart in a rectangular grid across the entire extent of either SII or the postcentral strip, and neuronal responses were sampled at 200-μm intervals through the depth of these cortical areas. The receptive fields of the neurons at the recording sites were determined by applying tactile stimulation at different locations on the contralateral body surface (*4*). Of the 11 hemispheres studied, 5 were intact and 6 had received lesions 6 to 8 weeks earlier of either the entire SII region (1 hemisphere) or selected portions of the body representations in the postcentral strip (Fig. 1A, 5 hemispheres) (*5*). The postcentral representations resected were those of all body parts (Fig. 1B, 1 hemisphere), of the hand only (Fig. 1C, 3 hemispheres), and of all body parts except the hand (Fig. 1D, 1 hemisphere). The resections were made by means of aseptic microsurgery while the animals were deeply anesthetized with Nembutal. Subsequent histological examination indicated that lesions and electrode tracks were located as planned.

In the SII cortex of the intact hemispheres, neurons with receptive fields for every part of the contralateral body were found readily, with most neurons having fields representing loci on the glabrous and hairy surfaces of the hand (Fig. 2A). In contrast, at 85 recording sites through SII in the case with a total removal of the postcentral strip (Fig. 1B), no neurons could be found that responded to tactile stimulation of any body part (Fig. 2B). That this total unresponsivity was not due simply to a general postoperative depression of the SII cortex, or to anesthetic effects, is indicated by the results in the other cases, which revealed highly selective somatic deactivations. Thus at 177 recording sites through SII of the three hemispheres in which only the postcentral hand representations had been removed (Fig. 1C), no neurons could be found that had receptive fields on the glabrous surface of the hand and only a few that had fields on the hand's hairy surface (Fig. 2A) (*6*), yet there was no difficulty in recording responses to stimulation of all other body parts. Similarly, at 322 recording sites distributed through SII in the case with a postcentral removal of the entire body representation except for the hand (Fig. 1D), all the responsive neurons found had receptive fields confined to the hand (Fig. 2D). In short, the elimination of any representation in the postcentral cortex eliminated that, and only that, representation in SII.

These results were for neuronal responses to light touch. If this low-amplitude stimulation did not activate an SII recording site in either the control or experimental hemispheres, that site was tested with the higher amplitude tactile stimulation afforded by a light tap. In the control hemispheres, the increase in stimulus intensity often activated previously unresponsive sites in SII and yielded a distribution of receptive fields similar to that illustrated for activation by light touch in these same hemispheres (Fig. 2A). In contrast, in the hemispheres with postcentral lesions, the high-amplitude stimulation did not activate any previously unresponsive site in SII, an indication that the absence of somatic responses of SII neurons was not due to an increase in their response threshold.

The functional dependency of SII on the postcentral cortex demonstrated by these results is not reciprocal. Thus in the control experiment in which we recorded across the postcentral cortex of the hemisphere from which the entire SII region had been removed, there was no obvious alteration of neuronal responsivity to tactile stimulation (*7*). Rather, neurons with receptive fields for all the major body parts were found easily and, furthermore, appeared to be as crisply responsive as those in the normal hemispheres.

In each of the cases with postcentral lesions, examination of Nissl-stained sections through the thalami ipsilateral to the removal revealed virtually total retrograde degeneration (*8*) in the somatotopically appropriate zone in VP (*9*). By contrast, in cases with SII lesions, there was no discernible degeneration in VP (*2*). These findings of thalamic degeneration agree with those from recent anatomical tracing studies in suggesting that VP sends at most a sparse input to SII. Nevertheless, whatever the density of such parallel inputs to SII from VP or any other nonpostcentral source, it is clear from our results that, in the absence of the postcentral strip and under our experimental conditions, the inputs are incapable of mediating somatic activation of SII neurons (*10*).

Recent anatomical evidence has revealed that neurons in each of the cytoarchitectonic fields of the postcentral strip, particularly pyramidal cells in layer III (*11*), project densely onto layer IV and the lower part of layer III in SII (*3*). This type of connection, from layer III of one cortical area to layers IV and lower III of another, has been shown in the visual system to be the specific route by which sensory information is transmitted

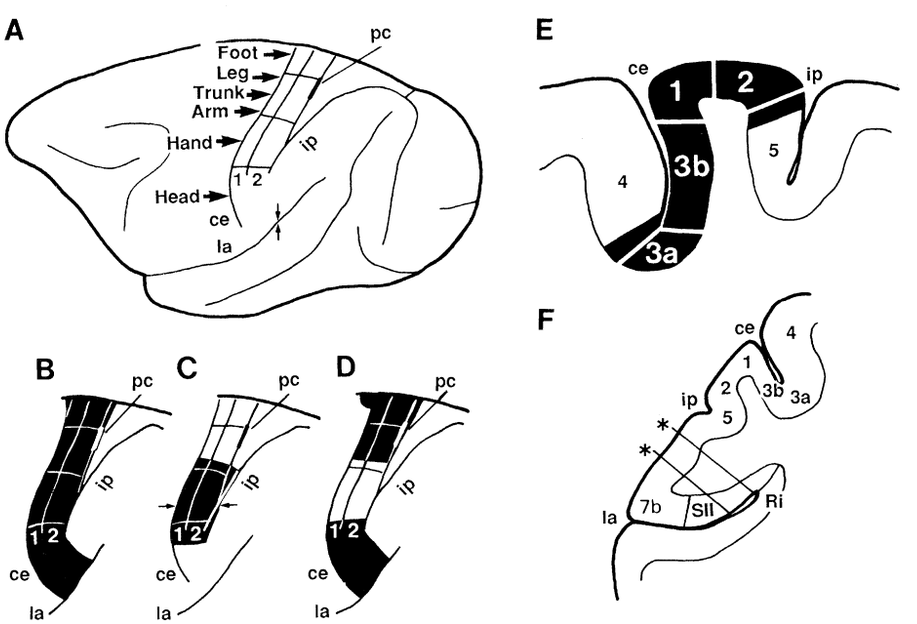

Fig. 1. (**A**) Dorsolateral view of a macaque brain, showing the approximate location of the representations of various body parts in the postcentral somatosensory strip. Areas 3a and 3b are located inside the central sulcus [see (E)] and are therefore not visible in this view, but their representations parallel those in areas 1 and 2. Vertical arrows show the location of the coronal section [illustrated in (F)]. (**B**, **C**, and **D**) The cortical regions removed in the three types of postcentral lesions are indicated in black. In each instance these lesions extended down the caudal bank of the central sulcus to include area 3b and into the fundus of the central sulcus to include area 3a [see (E)]. The lesions in (C) were extended 2.0 mm above and below the presumed borders of the hand representation to ensure its complete removal. Arrows in (C) indicate the location of the parasagittal section [shown in (E)]. (**E**) Parasagittal brain section through the hand representation of the postcentral strip, illustrating the location of the areas (3a, 3b, 1, and 2) that were removed [see (C)]. (**F**) A coronal section through SII, showing the location of SII and its bordering regions, areas 7b and Ri [see (A)]. The lines marked by asterisks indicate the plane of electrode penetrations through SII. Abbreviations: ce, central sulcus; ip, intraparietal sulcus; la, lateral sulcus; pc, postcentral sulcus; and Ri, retroinsular area. Numbers designate cytoarchitectural areas.

in serial fashion from earlier cortical stations to later ones (*12*). The present findings indicate that the same relation applies between the postcentral and SII portions of the somatosensory system and thus provide evidence outside the visual system that dense layer IV terminations of connections between cortical areas provide the anatomical basis for the forward flow of sensory information. Since this same laminar pattern of connections has also been found to link SII and the granular and dysgranular fields of the insula (*11*), it is likely that, after being activated by the postcentral cortex, SII in turn activates the insular cortex in a sequence analogous to the striate-prestriate-temporal sequence in vision. Indeed, our electrophysiological evidence provides support for the possibility (*13*) that all sensory modalities are served by such multisynaptic corticocortical sensory processing pathways.

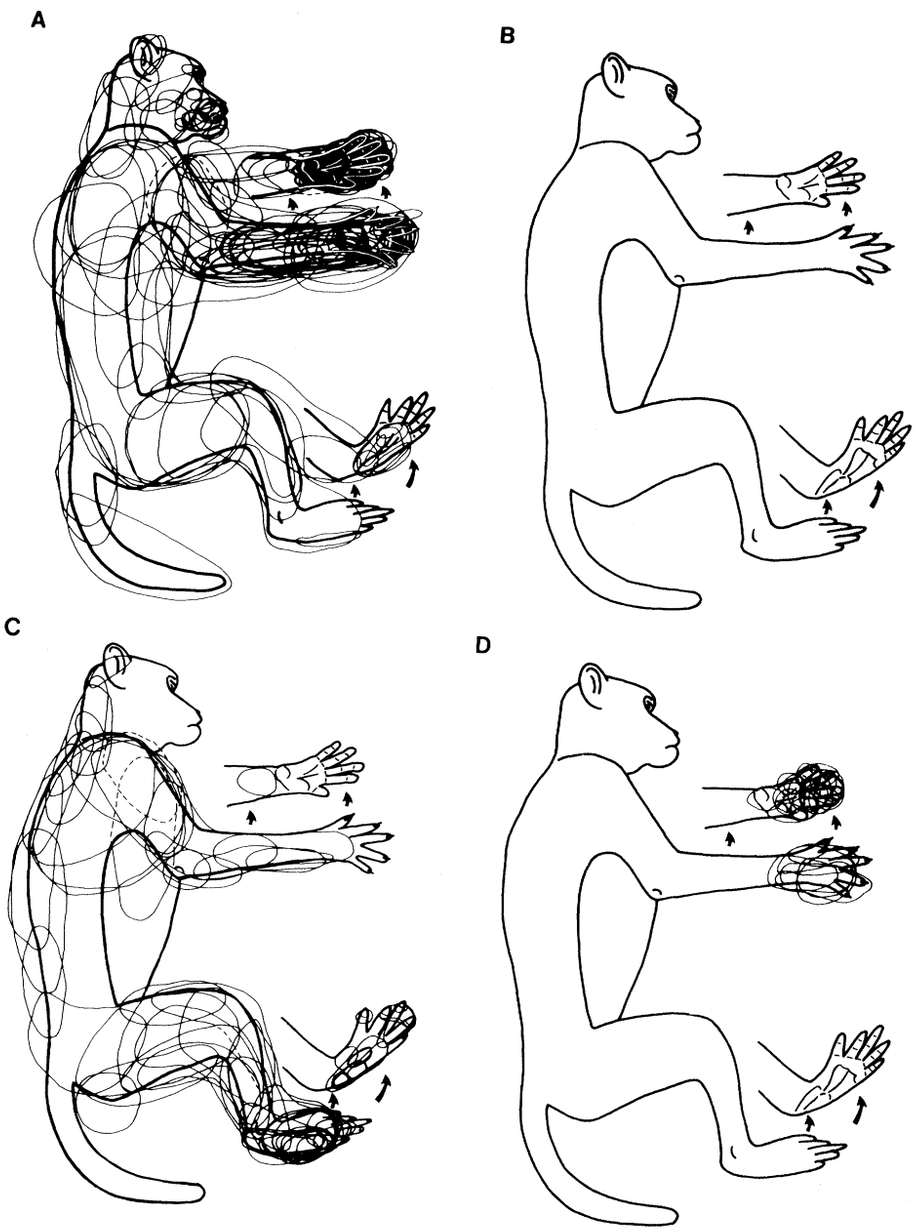

Fig. 2. (**A**) Composite drawing of all receptive fields found for SII neurons of three intact hemispheres in response to light tactile stimulation of the contralateral body surface. Note the high density of receptive fields for the hand. Receptive fields drawn outside the figure indicate extension of the fields onto an adjacent hidden surface. Bold arrows in this and other figures denote rotation of the body part (arm or leg) to allow visualization of its ventral surface. (**B**) Total absence of somatic receptive fields for SII neurons in the hemisphere with total ablation of the postcentral cortex (see Fig. 1B). (**C**) Total absence of receptive fields on the contralateral hand for SII neurons in one of the three hemispheres with lesions of the postcentral hand representations (see Fig. 1C). The relative decrease in the number of receptive fields for the head [compare with (A)] may reflect extension of the lesion of the hand representation into the locus of the head representation (see Fig. 1C). Conversely, the relative increase in the number of receptive fields for the trunk and foot [compare with (A)] may reflect a genuine postoperative increase in the size of those particular representations (*14*). (**D**) Receptive fields confined to the hand for SII neurons in the hemisphere with a postcentral lesion that spared only the hand representation (see Fig. 1D).

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6. This failure could not have been due to bias in electrode placement because most electrode penetrations through SII were aimed specifically at the representation of the hand (that is, at locations between the easily discovered representations of the head and foot). That we might have missed the hand representation by chance is even more unlikely, since this representation normally occupies 60 to 75% of the entire SII region, as estimated both from the present results and from the results of C. J. Robinson and H. Burton [*ibid.* **192**, 43 (1980)].
7. Since SII sends heavy projections to both supra- and infragranular layers of the postcentral strip, the loss of these projections presumably leads to some alteration in the properties of postcentral neurons [see, for example, J. H. Sandell and P. H. Schiller, *J. Neurophysiol.* **48**, 38 (1982)]. Any such changes, however, are apparently more subtle than could be detected with our methods.
8. Virtually complete degeneration of VP after postcentral lesions alone has not been reported before, perhaps because all the somatosensory tissue buried in the central sulcus, including area 3a as well as the most ventral parts of 3b (see Fig. 1E), had not previously been resected.
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10. We have considered two other possible explanations for our findings, but both appear to us to be highly unlikely. One possibility is that VP neurons projecting to the postcentral cortex send collaterals to activate SII neurons and that these collaterals are lost when VP neurons undergo retrograde degeneration. We now have evidence, however, that the SII hand representation is unresponsive on the day immediately after ablation of the postcentral hand representation, a postoperative period that is too short for the lack of response in SII to have been caused by the degeneration of any projection from VP. The second possibility is that our postcentral cortical lesions invaded the white matter below the depths of the central sulcus and, in doing so, interrupted thalamocortical fibers projecting to SII. This possibility seems remote, however, given the perfect correspondence between the representation resected in postcentral cortex and the representation deactivated in SII. For example, in the case in which the hand representation was spared, the cortex both lateral and medial to the hand representation was removed (Fig. 1D); that this divided lesion damaged thalamocortical fibers to SII serving both the face and the lower body, yet spared those between them serving the hand, seems highly improbable. Furthermore, removal of SII in the depths of the lateral sulcus, which had as much chance as the postcentral lesions of interrupting projections from VP coursing in the subcortical white matter, resulted neither in retrograde degeneration of VP nor in the somatic deactivation of the postcentral cortex.

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14. We have evidence that, after selective deactivation of the SII hand representation, the adjacent SII representations of the foot and trunk expand into the vacated territory.
15. Supported in part by NIH grants NS07704 and NS07497.

19 February 1987; accepted 28 April 1987

The Fragile X Site in Somatic Cell Hybrids: An Approach for Molecular Cloning of Fragile Sites

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Fragile X syndrome is a common form of mental retardation associated with a fragile site on the human X chromosome. Although fragility at this site is usually evident as a nonstaining chromatid gap, it remains unclear whether or not actual chromosomal breakage occurs. By means of somatic cell hybrids containing either a normal human X or a fragile X chromosome and utilizing two genes that flank the fragile site as markers of chromosome integrity, segregation of these markers was shown to be more frequent if they encompass the fragile site under appropriate culture conditions. Hybrid cells that reveal marker segregation were found to contain rearranged X chromosomes involving the region at or near the fragile site, thus demonstrating true chromosomal breakage within this area. Two independent translocation chromosomes were identified involving a rodent chromosome joined to the human X at the location of the fragile site. DNA analysis of closely linked, flanking loci was consistent with the position of the breakpoint being at or very near the fragile X site. Fragility at the translocation junctions was observed in both hybrids, but at significantly lower frequencies than that seen in the intact X of the parental hybrid. This observation suggests that the human portion of the junctional DNA may contain part of a repeated fragility sequence. Since the translocation junctions join heterologous DNA, the molecular cloning of the fragile X sequence should now be possible.

FRAGILE SITES ARE SPECIFIC CHROMOSOMAL loci, inherited in a Mendelian co-dominant manner, where chromatid gaps and/or breaks occur after specific biochemical induction. The molecular mechanism by which such sites exhibit fragility is unknown, although various genetic analyses have concluded that it is due to some, as yet unidentified, DNA sequence or sequences residing at the observed chromosomal site (*1*). Of the several human fragile sites currently recognized, the X chromosome site mapping to the vicinity of band Xq27.3 has been the most intensely studied as it is associated with mental retardation (*2*). This condition, referred to as fragile X–linked mental retardation or fragile X syndrome, is inherited in an X-linked semidominant fashion and is the single most common form of inherited mental deficiency in humans with a prevalence of greater than 1 per 2000 newborn males. It is an unusual X-linked disorder in that approximately 30% of carrier females express the phenotype of mental retardation and there is a relatively high frequency of nonpenetrant carrier males (*3*).

Chromosome breakage at the fragile X site, as well as at the autosomal sites, has been observed in individual metaphase spreads; questions have been raised as to whether chromosome breakage also occurs at fragile sites in the intact cell since physical shearing during cytogenetic preparation may influence chromatid stability at these sites (*4*). Furthermore, genetic evidence for breakage at fragile sites, such as their involvement in chromosome deletions or rearrangements, is not observed in appreciable frequencies. Although a correlation has been suggested between fragile site loci and the breakpoints of nonrandom chromosomal rearrangements in certain forms of cancer (*5*), significance of this association remains unclear, particularly in the absence of relevant data on fragile site breakage. We therefore wished to determine if there is a predilection for chromosome breakage at the fragile X site within the dividing cell and, if so, to determine if such breakage leads to useful rearrangements that could be utilized for subsequent molecular cloning experiments.

A strategy for using interspecific somatic cell hybrids to detect fragile site breakage was developed through the following rationale. First, we and others have shown that the fragile X site is cytogenetically expressed in hybrid cells and results in a cell system more easily manipulated for genetic analysis (*6*). Second, since there is evidence that the de novo loss of Xq28 (the region distal to the fragile site) in early embryogenesis leads to preferential X inactivation (*7*), it is possible that later somatic loss of this region on the fragile X chromosome results in mitotic failure and, hence, the absence of cytogenetic observations. A similar loss in a somatic cell hybrid would presumably be complemented by rodent loci and result in no significant effect on cell viability. Third, the experimental design could employ two X-linked loci which flank the fragile X site, those for hypoxanthine guanine phosphoribosyl transferase (HGPRT), and glucose-6-phosphate dehydrogenase (G6PD). By using rodent cells deficient in both activities fused with human cells, hybrids are isolated that express only the human forms of these enzymes. Since the HGPRT locus is proximal to the fragile X site relative to the centromere at Xq26–Xq27.2 (*8*), breakage in the vicinity of the fragile X site should result in an acentric fragment containing the G6PD locus at Xq28 (*8*) which would be mitotically unstable. It should then be possible to follow segregation of these two enzymes by placing positive selective pressure upon cells to maintain HGPRT activity followed by histochemical staining for G6PD activity.

Lymphoblasts, established from a clinically normal male or a retarded male with confirmed fragile X syndrome, were fused with Chinese hamster ovary cells deficient in both HGPRT and G6PD activities (*9*). Somatic cell hybrids expressing HGPRT were selected in medium containing hypoxanthine and azaserine [HAS medium; (*10*)] and determined to be expressing the human isomeric form of G6PD by electrophoresis. Two clones were selected for further analysis: Y75-1B, in which cytogenetic analysis revealed the presence of an intact fragile X chromosome as well as a human chromosome 13; and Y130-3A, which contained an intact X chromosome from a normal male and two unidentified human autosomes.

Colonies from either hybrid, grown in medium containing HAS, were consistently $G6PD^+$ (>90%) on the basis of in situ histochemical staining, which results in blue positive colonies and yellow negative colonies (*11*). Back-selection of the hybrids in

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