polylinker. This probe contains 197 bp of *src*-specific sequences that correspond to the Pst I–Ava I fragment shown on Fig. 3A. In the preparation of this probe, a slightly shorter fragment than the 220-bp-long Bam HI–Ava I probe was invariably observed. This fragment is most likely generated by Bam HI* activity. The probe was 5′ end-labeled at the Ava I site with T4 polynucleotide kinase and hybridized (20,000 count/min) for 16 hours at 56°C in 30 μl of S1 hybridization buffer (40m*M* Pipes, *p*H 6.4, 1 m*M* EDTA, 0.4*M* NaCl, and 50% formamide) [A. J. Berk and P. Sharp, *Cell* **12**, 1721 (1977)] to 30 μg of total RNA isolated from various mouse tissues. After the hybridization, 0.3 ml of ice-cold S1 buffer (0.25*M* NaCl, 0.05*M* Na acetate, *p*H 4.6, 4.5 m*M* $ZnSO_4$ and single-stranded carrier DNA at 20 μg/μl) containing 400 units of S1 nuclease/ml was added and single-stranded nucleic acids were digested for 90 minutes at 34°C. The S1 digestion was stopped by adding 50 μl of 4.0*M* ammonium acetate and 0.1*M* EDTA followed by a phenol/chloroform extraction, addition of 20 μg of transfer RNA, and isopropanol precipitation. The precipitate was dissolved in 0.2 ml of Na acetate (*p*H 5.6) and reprecipitated with ethanol. Finally the precipitate was dissolved in 6 μl of sequence loading buffer, boiled and size-separated on a 5% acrylamide, 7*M* urea sequencing gel.

21. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
22. The 3.8-kb λgt11 cDNA clone was cut with Nco I and the 1.8-kb fragment was isolated. The ends were filled in by means of the Klenow fragment of DNA polymerase and the fragment was blunt end–ligated to pUC18 previously digested with Bam HI and blunted with the Klenow fragment of DNA polymerase. This ligation recreates both the Bam HI site of pUC18 and the Nco I sites of the fragment. The resulting plasmid, pN1.8, was digested with Nco I and Sac I and the 1.4-kb c-*src*–containing fragment was isolated. In addition, pN1.8 was cut with Sac I and Bam HI and the 0.4-kb c-*src*–containing fragment was isolated. These fragments were ligated to a murine expression vector, pV5H, cut with Nco I and Bgl II to generate pVN1.8. pV5H consists of a Moloney murine leukemia virus backbone in which the chicken c-*src* coding sequence of p5H was cloned (B. Mathey-Prevot, unpublished). Digestion of pV5H with Nco I and Bgl II liberates the whole coding sequence of chicken c-*src* (see map of p5H) (*17*). pVN1.8 DNA was co-transfected with pSV2-Neo DNA [P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982)] into NIH/3T3 cells. Colonies were selected in the presence of G418 (1 mg/ml). Clones expressing $pp60^{c\text{-}src}$ were identified by immunoprecipitating cell lysates with the anti-$pp60^{v\text{-}src}$ monoclonal antibody Mab 327 (*4*). A positive clone, NIH/pVN1.8-p, was chosen for further studies. V8 mapping: NIH/3T3 and NIH/pVN1.8-p cultures were labeled with [^{32}P]orthophosphate (1 mCi/ml) for 4 hours. Cells were washed and lysed. Extracts were immunoprecipitated with 1 μl of Mab 327 and immunoprecipitates were electrophoresed on a 8.5% Laemmli SDS-polyacrylamide gel [U. K. Laemmli, *Nature (London)* **227**, 680 (1970)]. The $pp60^{c\text{-}src}$ bands were excised, treated with 100 ng of *Staphylococcus aureus* V8 protease as described [D. W. Cleveland *et al.*, *J. Biol. Chem.* **252**, 1102 (1977)], and separated on a 12.5% SDS-polyacrylamide gel. Following electrophoresis, the gel was dried and autoradiographed on XAR (Kodak) film with a lightning-plus screen.

23. We would like to thank Y. Citri for kindly providing the mouse brain cDNA library, J. Brugge for providing the Mab 327, and H. Hanafusa for providing p5H. We are also grateful to D. Fujita for having made his results available to us prior to publication and T. Wirth for reading the manuscript. Supported by an NIH grant (POI CA38497) to D.B. R.M. is a fellow of the American Cancer Society. B.M.-P. is a fellow of the Helen Hay Whitney Foundation. A.B. is a fellow of the European Molecular Biology Organization.

30 March, 1987; accepted 20 May 1987

The Molecular Basis of the Sparse Fur Mouse Mutation

GABOR VERES,* RICHARD A. GIBBS,† STEVEN E. SCHERER, C. THOMAS CASKEY

The ornithine transcarbamylase–deficient sparse fur mouse is an excellent model to study the most common human urea cycle disorder. The mutation has been well characterized by both biochemical and enzymological methods, but its exact nature has not been revealed. A single base substitution in the complementary DNA for ornithine transcarbamylase from the sparse fur mouse has been identified by means of a combination of two recently described techniques for rapid mutational analysis. This strategy is simpler than conventional complementary DNA library construction, screening, and sequencing, which has often been used to find a new mutation. The ornithine transcarbamylase gene in the sparse fur mouse contains a C to A transversion that alters a histidine residue to an asparagine residue at amino acid 117.

ORNITHINE TRANSCARBAMYLASE (OTC) is a mitochondrial matrix enzyme that catalyzes the conversion of ornithine and carbamyl phosphate to citrulline in the mammalian urea cycle (*1*). The enzyme is encoded by a single X-linked gene and is expressed in only two tissues, liver and intestine (*2*). Human OTC deficiency causes severe ammonia intoxication with accompanying metabolic disorders and neurological symptoms (*3*). Two mouse strains with an OTC mutation have been described—sparse fur (*spf*) (*4*) and sparse fur with abnormal skin and hair (spf^{ash}) (*5*). The *spf* mouse has an OTC with an overall decrease in activity, altered substrate affinity, a change in *p*H optima, and an increased amount of material that cross-reacts immunologically with an antibody to OTC (CRM) (*4, 6*).

Here we describe the molecular cloning and sequencing of the *spf* mouse OTC complementary DNA (cDNA) by the application of two recently described techniques—ribonuclease A (RNase A) cleavage (*7*) and the polymerase chain reaction (PCR) method for amplification of specific nucleotide sequences (*8*). In the past, the analysis of such a mutation would have required a substantial effort in both library construction and screening. However, the application of RNase A cleavage to localize the mutation followed by PCR amplification of the mutated site has greatly simplified this procedure.

To study the *spf* mutation, we first isolated the wild-type mouse OTC complementary DNA (cDNA) from a λgt11 library that was generated (*9*) from C57BL/6 mouse liver polyadenylated [poly(A)$^+$] RNA and screened with a partial rat OTC cDNA probe (*10*). The nucleotide sequence of the entire coding region and the 3′ untranslated region of the murine OTC cDNA are shown in Fig. 1. The protein coding regions of the mouse, rat, and human cDNA clones are the same length (1062 bases) and the murine gene is 96 and 88% homologous to the rat and human genes in this region, respectively. Most of the nucleotide substitutions are silent. The homology within the 3′ untranslated region of the murine gene is decreased to 80 and 62% of that of the rat and human sequences, respectively. Northern blot analysis indicated that the mouse OTC messenger RNA (mRNA) length is 1650 bases, which corresponds well with the rat and human (*11*) OTC mRNA size.

It was predicted from previous biochemical and enzymological studies that the *spf* mouse mutation could be a single point mutation (*6*), which was consistent with our observation that no differences in mRNA size could be seen between normal and *spf* mRNA by Northern blot analysis. To localize the *spf* mutation, we used RNase A cleavage (*7*) to characterize the OTC *m*RNA, thus avoiding the inherent problems of analyzing the large gene (approximately 60 kb) (*12*). A radiolabeled, antisense RNA probe was synthesized in vitro from the normal mouse OTC cDNA and then annealed to total RNA isolated from wild-type and *spf* mice. The samples were treated with RNase A to digest single-stranded RNA and to cleave any mismatches between the wild-type probe and the *spf* OTC mRNA. Wild-type RNA protected an approximately 1270-base probe fragment, which was the same length as the predicted length of homology with the OTC mRNA.

Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

*Permanent address: Hungarian Academy of Science, Biological Research Center, 6701 Szeged P.O.B. 521, Hungary.

†To whom correspondence should be addressed.

Table 1. Ornithine transcarbamylase activities in COS-cell extracts after transfection of wild-type and sparse fur murine OTC cDNAs cloned into the eukaryotic expression vector p91023(B) (*14*).

Mouse strain	Ornithine transcarbamylase activity (μmoles of citrulline/hour/ml of extract)		Ratio of activities (*p*H 10.0:*p*H 7.7)
	*p*H 7.7	*p*H 10.0	
Wild-type	12.9 ± 0.95	10.4 ± 1.0	0.81
Sparse fur	0.41 ± 0.045	4.81 ± 0.35	11.7

RNA from *spf* mice also protected a 1270-base fragment and, in addition, produced two distinct bands (920 and 350 bases) from partial RNase A cleavage of an internal mismatch site in the hybrids. Truncated RNA probes were used to unambiguously locate the RNase A cleavage site relative to the ends of the OTC mRNA (Fig. 2). A 5′ probe, which normally protected a 420-base fragment, was cleaved to 350 and 70 bases; this result indicated that the *spf* lesion was located approximately 350 bases downstream from the AUG codon in the *spf* OTC mRNA. The variation in cleavage efficiency of the two probes (Fig. 2, B and C) reflects the experimental variation that we find to be inherent in this assay.

To facilitate the molecular cloning of the region of the *spf* mouse mRNA containing the RNase A cleavage site we adapted the PCR amplification procedure (*8, 13*). A first strand cDNA was generated from poly(A)$^+$ *spf* RNA with random hexanucleotide primers and reverse transcriptase from avian myeloblastosis virus. Two synthetic 25-base oligonucleotides containing unique restriction endonuclease recognition sites were then used as specific primers to amplify a 420-bp segment of the cDNA corresponding to the 5′ end of the mRNA. After ten cycles of amplification, the PCR products were cleaved with the two appropriate restriction endonucleases and ligated directly into the polylinker region of an M13 DNA sequencing vector. Phage plaques were screened by hybridization with the labeled mouse OTC cDNA. The full 420-bp inserts from two

```
                    met leu ser asn leu arg ile leu leu asn asn ala ala leu arg lys gly his thr ser      20
mouse 5'      GAAG ATG CTG TCT AAT TTG AGG ATC CTG CTC AAC AAT GCA GCT CTT AGA AAG GGT CAC ACT TCT      64

val val arg his phe trp cys gly lys pro val gln ser gln val gln leu lys gly arg asp leu leu thr leu      45
GTG GTT CGA CAT TTT TGG TGT GGG AAG CCA GTC CAA AGT CAA GTA CAG CTG AAA GGC CGT GAC CTC CTC ACC TTG     139

lys asn phe thr gly glu glu ile gln tyr met leu trp leu ser ala asp leu lys phe arg ile lys gln lys      70
AAG AAC TTC ACA GGA GAG GAG ATT CAG TAC ATG CTA TGG CTC TCT GCA GAT CTG AAA TTC AGG ATC AAG CAG AAA     214

gly glu tyr leu pro leu leu gln gly lys ser leu gly met ile phe glu lys arg ser thr arg thr arg leu      95
GGA GAA TAT TTA CCT TTA TTG CAA GGG AAA TCC TTA GGA ATG ATT TTT GAG AAA AGA AGT ACT CGA ACA AGA CTG     289

ser thr glu thr gly phe ala leu leu gly gly his pro ser phe leu thr thr gln asp ile his leu gly val     120
TCC ACA GAA ACA GGC TTT GCT CTG CTG GGA GGA CAC CCT TCC TTT CTT ACC ACA CAA GAC ATT CAC TTG GGT GTG     364

asn glu ser leu thr asp thr ala arg val leu ser ser met thr asp ala val leu ala arg val tyr lys gln     145
AAT GAA AGT CTC ACA GAC ACC GCT CGT GTC TTA TCT AGC ATG ACA GAT GCA GTG TTA GCT CGA GTG TAT AAA CAA     439

ser asp leu asp thr leu ala lys glu ala ser ile pro ile val asn gly leu ser asp leu tyr his pro ile     170
TCA GAT CTG GAC ACC CTG GCT AAA GAA GCA TCC ATC CCA ATT GTC AAT GGA CTG TCA GAC TTG TAT CAT CCT ATC     514

gln ile leu ala asp tyr leu thr leu gln glu his tyr gly ser leu lys gly leu thr leu ser trp ile gly     195
CAG ATC CTG GCT GAT TAC CTT ACA CTC CAG GAA CAC TAT GGC TCT CTC AAA GGT CTT ACC CTC AGC TGG ATA GGG     589

asp gly asn asn ile leu his ser ile met met ser ala ala lys phe gly met his leu gln ala ala thr pro     220
GAT GGG AAC AAT ATC TTG CAC TCT ATC ATG ATG AGT GCT GCA AAA TTC GGG ATG CAC CTT CAA GCA GCT ACT CCA     664

lys gly tyr glu pro asp pro asn ile val lys leu ala glu gln tyr ala lys glu asn gly thr lys leu ser     245
AAG GGT TAT GAG CCA GAT CCT AAT ATA GTC AAG CTA GCA GAG CAG TAT GCC AAG GAG AAT GGT ACC AAG TTG TCA     739

met thr asn asp pro leu glu ala ala arg gly gly asn val leu ile thr asp thr trp ile ser met gly gln     270
ATG ACA AAT GAT CCA CTG GAA GCA GCA CGT GGA GGC AAT GTA TTA ATT ACA GAT ACT TGG ATA AGC ATG GGA CAA     814

glu asp glu lys lys lys arg leu gln ala phe gln gly tyr gln val thr met lys thr ala lys val ala ala     295
GAG GAT GAG AAG AAA AAG CGT CTT CAA GCT TTC CAA GGT TAC CAG GTT ACG ATG AAG ACT GCC AAA GTG GCT GCG     889

ser asp trp thr phe leu his cys leu pro arg lys pro glu glu val asp asp glu val phe tyr ser pro arg     320
TCT GAC TGG ACA TTT TTA CAC TGT TTG CCT AGA AAG CCA GAA GAA GTG GAT GAT GAA GTA TTT TAT TCT CCA CGG     964

ser leu val phe pro glu ala glu asn arg lys trp thr ile met ala val met val ser leu leu thr asp tyr     345
TCA TTA GTG TTC CCA GAG GCA GAG AAT AGA AAG TGG ACA ATC ATG GCT GTC ATG GTA TCC CTG CTG ACA GAC TAC    1039

ser pro val leu gln lys pro lys phe ***                                                               354
TCA CCT GTG CTC CAG AAG CCA AAG TTT TGA TGCCTGTCAAAAGGAAAAAAAACAGAAAACAAAACAATAACAATAACAACAACAACAACA  1127

AAAACCCCTCTGTTCTTTAGCAATAGAATAAGTCAGTTTATGTGGGAAAGAGAAGAATTTAAAATTGTAAACACATCCCTAGTGCATGGTATGATTATG  1224

TAATTGCTTTGCTATTATGAGAATTTCTTAAAGCTTTTAGTTTAAGTGCCTGGCATTTTATTATCCTGCTTGACTTGGTTTAAACACTCTCTTCAATTT  1318

ACAACCTCTGAATGACATTTGGGTATCATATTAATTATCATACACATTTCCTTCCACTAAACATTAAACACTTTGCTTACAATGTCTAAGTCATAAAAA20  1437
```

Fig. 1. Nucleotide and amino acid sequence of the mouse OTC cDNA. A complementary cDNA library was prepared in λgt11 phage, with oligo d(T)-primed double-stranded cDNA synthesized from female C57BL/6 mouse liver poly(A)$^+$ RNA (*9*), then ligated with linkers into the Eco RI site of the vector. A partial rat OTC cDNA clone was labeled by nick-translation and used to screen the mouse liver cDNA library. Approximately 10^5 phage were screened, of which three independent recombinant clones were found to hybridize to the probe. Inserts of these phage clones were isolated and subcloned into pUC9 and M13 vectors for further restriction analysis and sequencing. The complete sequence of the longest cDNA clone was determined by the dideoxynucleotide chain termination method (*15*). The predicted amino acid sequence of the mouse OTC is shown above the nucleotide sequence. The translation initiation codon is double underlined; the boxed amino acid and triplet code indicates the *spf* mutation (CAC → AAC) site. Underlined regions show the oligomer sequences used in the PCR reaction (*13*). The first primer at the 5′ end is complementary to the (−) strand and the second one is complementary to the (+) strand of the mouse OTC cDNA.

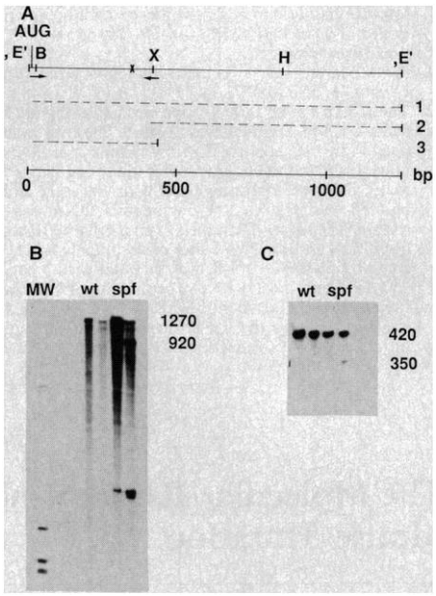

Fig. 2. RNase A cleavage analysis of wild-type and sparse fur mouse OTC RNA. (**A**) Diagram of the RNA probes used in the cleavage analysis. (**B**) Autoradiograph of the RNase A digestion products obtained with probe 1. Probe 2 provided full protection for both wild-type and *spf* RNA. (**C**) RNase cleavage pattern obtained with the 420-nucleotide 5′ probe 3. The smaller, 70-bp fragment, obtained with the *spf* mRNA, is not shown. The restriction sites are ,E′, Eco RI linkers; B, Bam HI; X, Xho I; H, Hind III. -X- is the site of the *spf* mutation. The arrows show the binding sites for the primers used in the PCR reaction. The RNase A cleavage assay was performed by a modification of the procedures of Myers *et al.* and Winter *et al.* (*7*). Fragments of the wild-type mouse OTC cDNA were subcloned into in vitro transcription vectors and RNA probes were generated from T7 polymerase-directed transcription in the presence of ^{32}P-GTP (650 Ci/mmol). Each probe (5×10^6 count/min) was hybridized to 200 μg of total cellular RNA and then the hybrids were partially purified by passage through polyuridylic acid–bound Messenger Affinity Paper (Amersham). Next, the hybrids were eluted, treated with RNase A (2 μg/ml and 10 μg/ml) at 25°C, and then analyzed on denaturing polyacrylamide gels. The autoradiograms in B and C represent 100 μg and 50 μg of total cellular RNA in each track, respectively. Size markers are shown at the left; sizes (in bases) are at the right.

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.

REFERENCES AND NOTES

1. S. Grisolia and P. P. Cohen, *J. Biol. Chem.* **198**, 561 (1952).
2. N. E. Jones, A. D. Anderson, C. Anderson, S. Hodes, *Arch. Biochem. Biophys.* **95**, 499 (1961).
3. M. Walser, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, M. S. Brown, Eds. (McGraw-Hill, New York, ed. 5, 1983), pp. 402–438.
4. R. DeMars, S. L. LeVan, B. L. Trend, L. B. Russell, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1693 (1976).
5. D. P. Doolittle, L. L. Hulbert, C. Cordy, *J. Hered.* **65**, 194 (1974).
6. P. Briand, B. François, D. Rabier, L. Cathelineau, *Biochim. Biophys. Acta* **704**, 100 (1982); I. A. Qureshi, J. Letarte, R. Ouellet, *Pediat. Res.* **13**, 807 (1979); *J. Pediat. Gast. Nutrit.* **4**, 118 (1985).
7. R. M. Myers, Z. Larin, T. Maniatis, *Science* **230**, 1242 (1985); E. Winter, F. Yamamoto, C. Almoguera, M. Perucho, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7575 (1985); R. A. Gibbs and C. T. Caskey, *Science* **236**, 303 (1987); F. F. Chehab, G. R. Honig, Y. W. Kan, *Lancet* **1986-I**, 3 (1986).
8. F. A. Faloona and K. B. Mullis, *Methods Enzymol.*, in press; R. K. Saiki, T. L. Bugawan, G. T. Horn, K. B. Mullis, H. A. Erlich, *Nature (London)* **324**, 163 (1986); R. K. Saiki *et al.*, *Science* **230**, 1350 (1985).
9. R. A. Young and R. W. Davies, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983).
10. The rat OTC cDNA probe was kindly supplied by R. Nussbaum.
11. A. L. Horwich *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4258 (1983); A. L. Horwich *et al.*, *Science* **224**, 1068 (1984).
12. S. Scherer and G. Veres, manuscript in preparation.
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.
15. M. O. Biggin, T. J. Gibson, G. F. Hong, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3963 (1983).

16. G.V. was supported by grant Q-533 from the Robert A. Welch Foundation, R.A.G. is supported by a postdoctoral fellowship from the Muscular Dystrophy Association, S.E.S. supported by NIH grant HD21452, and C.T.C. is an investigator with the Howard Hughes Medical Institute. We thank J. Chamberlain for critical reading of the manuscript, Grant MacGregor for useful suggestions and the gift of pRSVZ, Donna Muzny for technical assistance, and J. Habener for synthesizing the oligonucleotide primers.

23 December 1986; accepted 27 May 1987

Physiological Evidence for Serial Processing in Somatosensory Cortex

T. P. PONS,* P. E. GARRAGHTY, D. P. FRIEDMAN, M. MISHKIN

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-

T. P. Pons and M. Mishkin, Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.
P. E. Garraghty, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.
D. P. Friedman, Neurosciences Research Branch, National Institute on Drug Abuse, Rockville, MD 20857.

*To whom correspondence should be addressed.