of calpain II supports the idea that location of calpain II may be critical to the mode of calpain II function in terms of access to substrate or mode of activation or deactivation.

Clearly, the nature of the interrelationship between calpain II and calpastatin is not yet understood. For instance, we do not yet know whether the mechanism by which calpastatin controls calpain II relies more heavily on (i) a physical association that necessitates physical proximity [sarcolemmal association of calpastatin and calpain II in striated muscle (*11*, *12*)] or whether (ii) the Ca^{2+} microenvironment may be more important in modulating the calpain II–calpastatin interaction. Injected calpain I, though inhibited during in vitro interactions with calpastatin, does not relocate during mitosis and does not appear to affect the progress of specific mitotic states (*13*). This result provides additional support for the theory that relocation of calpain II may be critical to how the protease functions in mitosis.

Insight into mechanisms by which the Ca^{2+} requirement of calpain II may be modulated has come from calpain activation studies in smooth muscle (*14*). The amount of Ca^{2+} required for the autolysis of half of a total amount of calpain II ($K_{0.5}$) can be reduced from 680 μM to 87 μM if phosphatidylinositol or diacylglyerol are added. However, these Ca^{2+} levels are still substantially greater than those reported at metaphase-anaphase transition (0.5 to 0.8 μM) (*4*) for PtK_1 cells. Additional work needs to address the potential involvement of Ca^{2+}-binding proteins in combination with other factors in the modulation of this protease.

Although one of the original reasons for the use of microinjection was to facilitate the identifications of cellular substrates, there may be an array of substrates for calpain II. Hydrolysis of specific cytoskeletal proteins (*15*, *16*) as well as microtubule-associated proteins (MAPs) (*17–19*) by calpain II has been well documented. Calpain II–promoted hydrolysis of MAP 1 or MAP 2 could result in the disassembly of the microtubules of the mitotic spindle. Moreover, the selective hydrolysis of filamin and other actin-binding proteins (*20*, *21*), as well as hydrolysis of intermediate filament components (*22*), has major implications for any of the mitotic processes dependent on these cytoskeletal components. The possible role that calpain II may play in association with the nucleus, nuclear membrane, or chromosomes is somewhat more speculative. Calpain II has been implicated in the Ca^{2+}-mediated intracellular processing of various receptors that become associated with the nucleus (*23*, *24*), as well as in the cytoskeletal-directed condensation of chromosomes (*25*).

REFERENCES

1. C. H. Keith, F. R. Maxfield, M. L. Shelanski, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 800 (1985).
2. C. H. Keith, R. Ratan, F. R. Maxfield, A. Bajer, M. L. Shelanski, *Nature (London)* **316**, 848 (1985).
3. P. K. Hepler and S. M. Wolniak, *Int. Rev. Cytol.* **90**, 169 (1984).
4. M. Poenie, J. Alderton, R. Steinhardt, R. Tsien, *Science* **233**, 886 (1986).
5. M. J. Welsh, J. R. Dedman, B. R. Brinkley, A. R. Means, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1867 (1979).
6. V. L. Smith, T. L. Reed, J. Mathew, J. R. Dedman *J. Cell. Biol.* **103**, 35/a (1986).
7. D. P. Kiehart, *ibid.* **88**, 604 (1981).
8. J. G. Izant, *Chromosoma (Berlin)* **88**, 1 (1983).
9. M. Schliwa, U. Enteneuer, J. C. Bulinski, J. G. Izant, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1037 (1981).
10. W. R. Dayton, D. E. Goll, M. D. Zeece, R. M. Robson, W. J. Reville, *Biochemistry* **15**, 2150 (1976).
11. R. L. Mellgren, R. D. Lane, S. S. Kakar, *Biochem. Biophys. Res. Commun.* **142**, 1025 (1987).
12. W. R. Dayton and J. E. Schollmeyer, *Exp. Cell. Res.* **136**, 423 (1981).
13. J. E. Schollmeyer, in preparation.
14. S. A. Colligan and D. R. Hathaway, *J. Biol. Chem.* **259**, 11627 (1984).
15. S. Pontremoli and E. Melloni, *Annu. Rev. Biochem.* **55**, 455 (1986).
16. T. Murachi, *Biochem. Soc. Symp.* **49**, 149 (1983).
17. I. V. Sandoval and K. Weber, *Eur. J. Biochem.* **92**, 463 (1978).
18. I. Klein, D. Lehotay, M. Gondek, *Arch. Biochem. Biophys.* **208**, 520 (1981).
19. C. Sato *et al.*, *Cell. Struct. Funct.* **11**, 253 (1986).
20. P. J. A. Davies *et al.*, *J. Biol. Chem.* **253**, 4036 (1978).
21. N. C. Collier and K. Wang, *ibid.* **257**, 6937 (1982).
22. W. J. Nelson and P. Traub, *Mol. Cell. Biol.* **3**, 1146 (1983).
23. R. E. Gates and L. E. King, Jr., *Biochem. Biophys. Res. Commun.* **113**, 255 (1983).
24. A. Murayama, F. Fukai, T. Murachi, *Biochemistry* **95**, 1697 (1984).
25. M. Hayashi, M. Inomata, M. Nakamera, K. Imahori, S. Kawashima, *J. Biochem.* **17**, 1363 (1985).
26. B. H. Devlin and I. Konigsberg, *Dev. Biol.* **95**, 175 (1983).
27. J. E. Schollmeyer, *Exp. Cell Res.* **163**, 413 (1986).
28. M. Zavotink, M. J. Welsh, J. R. McIntosh, *ibid.* **149**, 375 (1983).
29. A. Graessmann, M. Graessmann, C. Mueller, *Methods Enzymol.* **65**, 816 (1980).
30. R. A. Lepley, M. Pampusch, W. R. Dayton, *Biochem. Biophys. Acta* **828**, 95 (1985).

23 October 1987; accepted 15 March 1988

Identification and Characterization of a Neuron-Specific Nuclear Antigen in *Drosophila*

ETHAN BIER, LARRY ACKERMAN, SANDRA BARBEL, LILY JAN, YUH NUNG JAN

An antigen found only in neuronal nuclei of *Drosophila melanogaster* is revealed by staining with a monoclonal antibody (Mab44C11). This antigen appears early in development, before neurons show any other signs of antigenic or morphologic differentiation, and persists throughout development. This nuclear staining permits reliable detection of neurons in developmental studies of wild-type and mutant flies. Protein immunoblot analyses and immune precipitation experiments show that the neuronal nuclear antigen is a 50-kilodalton polypeptide.

THE DIFFERENCE BETWEEN NEURONS and other types of cells remains a major question in developmental neurobiology. In *Drosophila*, this problem can be studied by two complementary approaches. (i) From mutational studies, one can identify genes that are important in neuronal determination (*1–3*). (ii) With neuron-specific antibodies, one can identify molecular differences between neurons and other cell types. Although a number of antibodies have been found to stain subsets of cells of the nervous system (*4*), only one antiserum, raised against horseradish peroxidase (HRP), was previously known to stain all *Drosophila* neurons [antibodies to HRP bind to carbohydrate epitopes on the neuronal membrane (*5–7*)]. Here we describe a monoclonal antibody (Mab44C11) that exclusively stains neuronal nuclei in *Drosophila*. We also present preliminary biochemical characterizations of this neuron-specific nuclear antigen.

The antibody Mab44C11 was found as an immunoglobulin M (IgM)–secreting clone from a hybridoma fusion with HRP as an immunogen; however, it does not recognize HRP. At all stages of *Drosophila* development that have been examined, Mab44C11 stains neuronal nuclei. In the central nervous system (CNS), nuclei of neurons but not neuroblasts are stained. Although it appears that all neurons are labeled, because of the high density of cells we could not be certain that every neuron in the CNS is stained. In the embryonic peripheral nervous system (PNS), on the other hand, the number and position of all neurons has been determined (*8*). Examination of many embryos revealed

Howard Hughes Medical Institute and the Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94143.

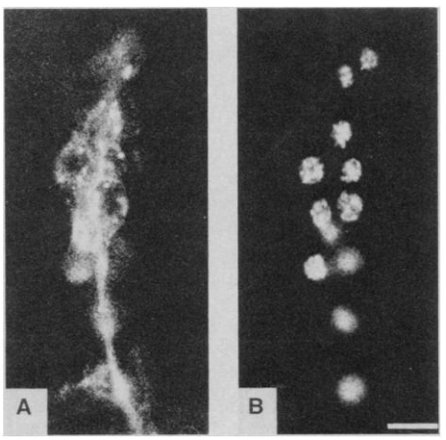

Fig. 1. One-to-one correspondence of cells labeled with (**A**) antibodies to HRP and with (**B**) Mab44C11 in a dorsal cluster of peripheral neurons in an abdominal segment of a stage 16 embryo. In this double-labeling experiment, antibodies to HRP were labeled with rhodamine-conjugated goat antibodies to rabbit IgG, and Mab44C11 was labeled with fluorescein-conjugated goat antibodies to mouse IgM. Bar, 20 μm.

that all neurons but no other cells in the periphery express this nuclear antigen. A one-to-one correspondence is found between cells stained with antibodies to HRP and with Mab44C11 (Fig. 1).

In both the CNS and PNS the Mab44C11 antigen appears as soon as or shortly after a neuron is born and is the earliest neuron-specific antigen of which we are aware. In *Drosophila*, embryogenesis lasts for about 22 hours at 25°C. The Mab44C11 staining reveals the first peripheral neuron at stage 11 [approximately 7 hours after fertilization; staging is according to (*9*)] (Fig. 2A). During the next 3 hours, staining of other peripheral neurons appears in a stereotyped pattern (Fig. 2, B to D). In contrast, staining by antibodies to HRP (*5*) or Mab21A4, which recognizes all sensory neurons (*10*), first appears early during stage 13 (about 9.5 hours after fertilization). Axonogenesis also begins at stage 13, and by stage 14 (10 hours after fertilization) all neurons are present and express a number of neuron-specific antigens (Fig. 2, D to F). After embryogenesis, a second wave of neurogenesis takes place and gives rise to the adult nervous system. For instance, adult sensory neurons start to appear in the imaginal disks during the late third instar larval and early pupal stages (*10*). One of the first signs of neuronal differentiation in these cells is nuclear staining by Mab44C11. The Mab44C11 antigen persists throughout the development of larval and adult neurons.

The early onset and nuclear localization of this neuron-specific antigen make it an excellent marker for analyzing mutations affecting neural development. To illustrate this point we show a Notch mutant with an overall increase of both central and peripheral neurons (*1*) and a scute mutant with a severe reduction of central and peripheral neurons (*11*) (Fig. 3). Compared to staining of neuronal surface or cytoplasm, the Mab44C11 staining of neuronal nuclei permits easy, unambiguous identification of individual neurons. This resolution is essential in counting neurons in large clusters.

As a first step in the biochemical study of the Mab44C11 antigen, we looked for proteins that bound specifically to this antibody. On protein immunoblots (*12*), two bands (at 21 and 50 kD) were seen only in embryos that had begun neurogenesis (older than 6 hours) and showed Mab44C11 staining (Fig. 4A). A 45-kD band, common to all embryos (Fig. 4A) and comigrating with one major yolk protein band, was also stained by other monoclonal antibodies in control experiments and was probably nonspecific. Because extracts of embryonic and larval CNS and adult heads gave rise to only the 50-kD immunoreactive band (Fig. 4A) and immune precipitation experiments described below also revealed the 50-kD band but not the 21-kD band, the 50-kD band is the only suitable candidate for the cytochemical antigen.

In addition to tissues and developmental stages described above, 2- to 4-day pupae also contain the 50-kD band. This immunoreactive band is present at much lower amounts in third instar larvae and adult male abdomens, where nervous tissues represent only a small proportion of the total mass. Thus, the level of the 50-kD immunoreactive band correlates with the amount of nervous tissue.

As additional controls, we used mutant embryos with reduced nervous tissue, embryos of distantly related *Drosophila* species, as well as other monoclonal antibodies. Since the CNS is derived from ventrolateral regions of the embryo, it should be greatly reduced in embryos of homozygous dorsalized (*dl*) mothers; these mutant embryos fail to develop ventral tissues (*13*). Indeed, Mab44C11 staining revealed no neurons in most mutant embryos and small clusters of stained cells in the rest. Consistent with the sparse staining, little of the 50-kD band is found in these *dl* embryos (Fig. 4A). Embryos from *Drosophila virilis*, which had diverged from *Drosophila melanogaster* 50 to 60 million years ago, do not stain histochemically, nor do they show any bands on protein immunoblots (Fig. 4A). Control blots (not shown) of early embryos (0 to 6 hours), late embryos (6 to 18 hours), and dissected larval CNS were probed with three other IgM class monoclonal antibodies (specific for pole cells, body wall muscle, or a general nuclear antigen). No 50-kD band was revealed. Therefore, a strong correlation was found between the 50-kD band and Mab44C11 staining of neurons.

In immunoblotting, the proteins are denatured and bound to nitrocellulose paper

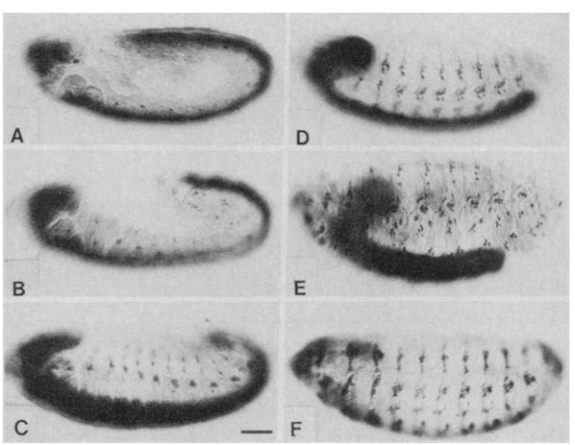

Fig. 2. Appearance of peripheral neurons in embryos as revealed by Mab44C11 staining (**A** to **E**) and by Mab21A4 staining (**F**). (A) A late stage 11 embryo showing one or two cells labeled with Mab44C11 in each hemisegment. (B) A late stage 12 embryo showing Mab44C11 staining of cells in the lateral cluster after staining is apparent in the dorsal cluster. (C to E) Embryos at stages 13, 14, and 16, respectively, stained with Mab44C11. In (E) the full complement of peripheral neurons is shown. (F) A stage 15 embryo with fully developed peripheral nervous system, labeled with Mab21A4 (*10*). Bar, 200 μm.

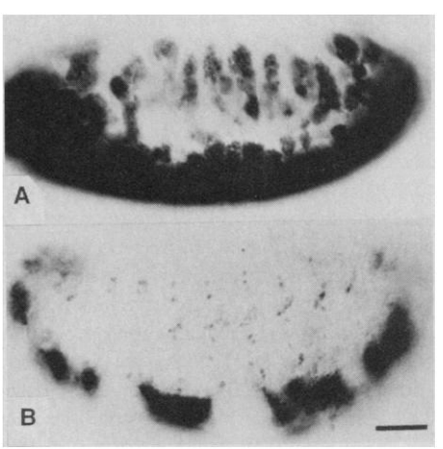

Fig. 3. Examples of mutant phenotypes in the nervous system seen with Mab44C11 staining. (**A**) A Notch mutant (*Df(1) N*^{8}) embryo with severe hypertrophy of both CNS and PNS. (**B**) A scute mutant (*sc*^{B57}) embryo with reduced numbers of CNS and PNS neurons. Bar, 200 μm.

before exposure to the antibody. To determine whether the 50-kD protein binds to Mab44C11 in solution, we used this antibody to precipitate the antigen from ^{35}S-labeled CNS. A 50-kD protein was precipitated (Fig. 4B) (but not by a control monoclonal antibody) and shown in other experiments to comigrate with the 50-kD band on protein immunoblots. Moreover, after precipitation of unlabeled embryonic protein by Mab44C11, a 50-kD protein can be eluted that is recognized by Mab44C11 on immunoblot (Fig. 4C). In this experiment most proteins with a molecular size of 50 kD are removed during immune precipitation. Therefore, the 50-kD band on protein immunoblots is most likely due to specific binding rather than cross-reactivity of a major protein. Taken together, both protein immunoblot and immune precipitation experiments strongly suggest that the Mab44C11 antigen is at least in part made up of a 50-kD protein.

A number of nuclear proteins have been characterized. Notable examples are some of the segmentation and homeotic gene products, which show position-dependent distribution and play crucial roles in pattern formation and gene regulation (*14*). Mab44C11 antigen is the first nuclear antigen known to have tissue type–dependent distribution. Although its function in neural development is not known, we have found a connection between this antigen and the embryonic lethal abnormal visual system (*elav*) locus (*3*) whose embryonic transcripts are limited to the nervous system (*15*). Deletions or lethal point mutations of the *elav* locus eliminate Mab44C11 staining, although neurons can be visualized in these mutant embryos by staining with antibodies to HRP. From our studies (*16*) we cannot be sure whether the *elav* locus encodes the Mab44C11 antigen or controls its expression; further characterization of the *elav* gene products (*15*) should resolve this question. Studies of the function of the Mab44C11 antigen and of the neuron-specific expression of this nuclear protein may provide insight to the molecular mechanisms that differentiate neurons from other types of cells.

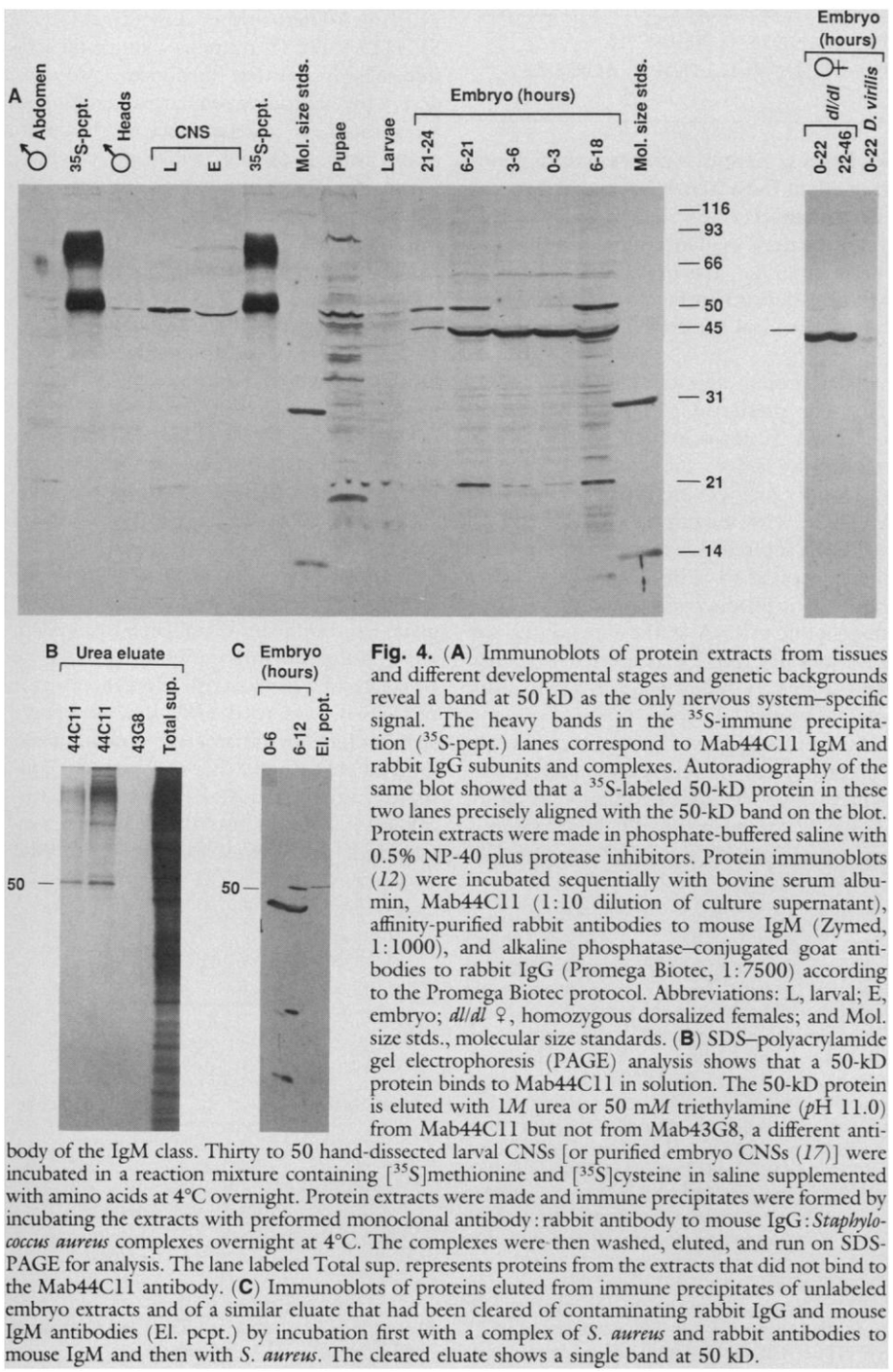

Fig. 4. (**A**) Immunoblots of protein extracts from tissues and different developmental stages and genetic backgrounds reveal a band at 50 kD as the only nervous system–specific signal. The heavy bands in the ^{35}S-immune precipitation (^{35}S-pcpt.) lanes correspond to Mab44C11 IgM and rabbit IgG subunits and complexes. Autoradiography of the same blot showed that a ^{35}S-labeled 50-kD protein in these two lanes precisely aligned with the 50-kD band on the blot. Protein extracts were made in phosphate-buffered saline with 0.5% NP-40 plus protease inhibitors. Protein immunoblots (*12*) were incubated sequentially with bovine serum albumin, Mab44C11 (1:10 dilution of culture supernatant), affinity-purified rabbit antibodies to mouse IgM (Zymed, 1:1000), and alkaline phosphatase–conjugated goat antibodies to rabbit IgG (Promega Biotec, 1:7500) according to the Promega Biotec protocol. Abbreviations: L, larval; E, embryo; *dl/dl* ♀, homozygous dorsalized females; and Mol. size stds., molecular size standards. (**B**) SDS–polyacrylamide gel electrophoresis (PAGE) analysis shows that a 50-kD protein binds to Mab44C11 in solution. The 50-kD protein is eluted with 1*M* urea or 50 m*M* triethylamine (*p*H 11.0) from Mab44C11 but not from Mab43G8, a different antibody of the IgM class. Thirty to 50 hand-dissected larval CNSs [or purified embryo CNSs (*17*)] were incubated in a reaction mixture containing [^{35}S]methionine and [^{35}S]cysteine in saline supplemented with amino acids at 4°C overnight. Protein extracts were made and immune precipitates were formed by incubating the extracts with preformed monoclonal antibody : rabbit antibody to mouse IgG : *Staphylococcus aureus* complexes overnight at 4°C. The complexes were then washed, eluted, and run on SDS-PAGE for analysis. The lane labeled Total sup. represents proteins from the extracts that did not bind to the Mab44C11 antibody. (**C**) Immunoblots of proteins eluted from immune precipitates of unlabeled embryo extracts and of a similar eluate that had been cleared of contaminating rabbit IgG and mouse IgM antibodies (El. pcpt.) by incubation first with a complex of *S. aureus* and rabbit antibodies to mouse IgM and then with *S. aureus*. The cleared eluate shows a single band at 50 kD.

REFERENCES AND NOTES

1. J. A. Campos-Ortega, *Trends Neurosci.* **8**, 245 (1985); Y. Yedvobnick *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 841 (1985).
2. R. Bodmer *et al.*, *Cell* **51**, 293 (1987).
3. A. Campos, D. Grossman, K. White, *J. Neurogenet.* **2**, 197 (1985).
4. M. J. Bastiani, A. L. Harrelson, P. M. Snow, C. S. Goodman, *Cell* **48**, 745 (1987); S. L. Zipursky, T. R. Venkatesh, D. B. Teplow, S. Benzer, *ibid.* **36**, 15 (1984); S. L. Zipursky, T. R. Venkatesh, S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1855 (1985).
5. L. Y. Jan and Y. N. Jan, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2700 (1982).
6. P. Snow, N. Patel, A. Harelson, C. Goodman, *J. Neurosci.* **7**, 4137 (1987).
7. F. Katz, W. Moats, Y. N. Jan, in preparation.
8. V. Hartenstein and J. A. Campos-Ortega, *Roux's Arch. Dev. Biol.* **195**, 210 (1986); A. Ghysen, C. Dambly-Chaudiere, E. Aceves, L. Y. Jan, Y. N. Jan, *ibid.*, p. 281; R. Bodmer and Y. N. Jan, *ibid.* **196**, 69 (1987).
9. J. A. Campos-Ortega and V. Hartenstein, *The Embryonic Development of* Drosophila melanogaster (Springer-Verlag, Berlin, 1985).
10. Y. N. Jan, A. Ghysen, I. Christoph, S. Barbel, L. Y. Jan, *J. Neurosci.* **5**, 2453 (1985).
11. C. Dambly-Chaudiere and A. Ghysen, *Genes Dev.* **1**, 297 (1987).
12. J. M. Gershoni and G. E. Palade, *Anal. Biochem.* **131**, 1 (1983). The electrophoresis buffers and gels were made according to Laemmli [V. Laemmli, *Nature* **227**, 680 (1970)].
13. K. Anderson and C. Nusslein-Volhard, in *Gametogenesis and the Early Embryo*, J. G. Gall, Ed. (Liss, New York, 1986), pp. 177–194.
14. W. J. Gehring, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 243 (1985).
15. A. R. Campos, D. C. Rosen, S. N. Robinow, K. White, *EMBO J.* **6**, 425 (1987).
16. The absence of Mab44C11 staining in mutant *elav* embryos suggests that the *elav* gene either codes for the Mab44C11 antigen or that the *elav* gene product is required for the expression of the Mab44C11 antigen. If the former is true, one might expect larvae or flies with more copies of *elav* to have more Mab44C11 antigen per total protein than those with fewer copies. This possibility was tested by mating wild-type female flies to males that carried an X chromosome deficient for *elav* and a duplication of

the *elav* locus on the Y chromosome. Male progeny of this cross should behave (because of dosage compensation) as though four haploid *elav* copies were present, and female progeny should be haploid. Male and female larval CNS and adult heads were dissected separately, and the amount of Mab44C11 antigen was assayed by protein immunoblotting of serial dilutions of the protein extracts. We found that males and females have equal amounts of the Mab44C11 antigen. Thus, at present no conclusive evidence exists to suggest that *elav* is the structural gene for the Mab44C11 antigen.

17. J. Thomas, personal communication.

18. Supported by the Howard Hughes Medical Institute and NIH grant NS19191. We thank K. White for sending us *elav* mutants and L. Schulte for help in preparing the manuscript.

13 November 1987; accepted 11 March 1988

HTLV-II Transactivation Is Regulated by the Overlapping *tax/rex* Nonstructural Genes

JOSEPH D. ROSENBLATT, ALAN J. CANN, DENNIS J. SLAMON, IRA S. SMALBERG, NEIL P. SHAH, JOYCE FUJII, WILLIAM WACHSMAN,* IRVIN S. Y. CHEN†

The human T-cell leukemia virus (HTLV) types I and II have two nonstructural genes that are encoded in overlapping reading frames. One of these genes, known as *tax*, has been shown to encode a protein responsible for enhanced transcription (transactivation) from the viral long terminal repeats (LTRs). Genetic evidence indicates that the second nonstructural gene of HTLV-II, here designated *rex*, acts in trans to modulate *tax* gene–mediated transactivation in a concentration-dependent fashion. The *rex* gene may regulate the process of transactivation during the viral life cycle.

HTLV-I AND HTLV-II ARE ASSOciated with specific T-cell disorders in humans (*1–3*). HTLV-I, HTLV-II, and the related bovine leukemia virus (BLV) each have two genes located in the 3′ region of the genome that encode nonstructural proteins (Fig. 1) (*4–6*). One of these genes, called *tax* (which has also been referred to as *x-lor*, *tat*-1, *tat*-2, and *x*), encodes a protein, designated $p40^{xI}$ in HTLV-I and $p37^{xII}$ in HTLV-II (*4*). The *tax* gene product is required for HTLV replication (*7*), and acts in trans to increase the rate of viral transcription from the viral LTR (*8–11*). The second gene is encoded in an alternate open reading frame that partially overlaps with the *tax* gene on the same subgenomic mRNA (Fig. 1A) (*5, 12, 13*). For HTLV-I, the second nonstructural gene, called *tel* (for trigger for expression of late genes) (*14*) or *rex* (for regulator of expression) (*15*) encodes a major protein species of 27 kD, $p27^{xIII}$, which is localized to the nucleus of HTLV-I–infected cells, as well as a 21-kD protein species $p21^{xIII}$ (*5, 12, 14*). The $p27^{xIII}$ protein of HTLV-I is required for efficient *gag* expression (*14*).

The corresponding *rex* gene of HTLV-II encodes protein species of 26 and 24 kD (*13*). The presumed Met initiation codon for HTLV-II *rex* is located on the middle exon of the 2.1-kb subgenomic mRNA at nucleotide (nt) position 5121 (Fig. 1A) (*13*). The Met initiation codon for $p37^{xII}$ (AUG) is located downstream in the same middle exon at nt 5180 (*16*). The fact that the *rex* gene product is encoded by the same subgenomic mRNA as the trans-acting *tax* gene product, $p37^{xII}$, suggested that, like *tax*, the *rex* gene might also play a regulatory role in the viral life cycle.

To express, HTLV-II viral genes in vitro, we constructed the expression vector, SV-HTLV (Fig. 1B) which contains the entire HTLV-II coding region (nt 361 to 8550) under the control of the SV40 promoter (*8*). Co-transfection of SV-HTLV with the HTLV-II LTR into COS cells results in transactivation of the HTLV-II LTR, as measured by enhanced expression of LTR-linked indicator genes. LTR function was assayed with the LTR-II-CAT construct, in which the bacterial chloramphenicol acetyltransferase (CAT) gene is linked to the HTLV-II LTR (*8*).

Two mutations were introduced into SV-HTLV that specifically prevent wild-type *rex* expression (Fig. 1B). Mutant SV-HTLV-Sph has a 4-bp deletion that eliminates the Met initiation condon for *rex*, but does not affect the *tax* initiation codon, which is located further downstream (*17*). SV-HTLV-rex^{term} contains a single nucleotide substitution that introduces a stop codon in the *rex* coding sequences, resulting in a truncated *rex* gene product of 79 amino acids instead of the wild-type product, which is 170 amino acids (*17*) (Fig. 1B). Co-transfection of LTR-II-CAT into COS cells with either the SV-HTLV-Sph or SV-HTLV-rex^{term} mutants defective for *rex* expression results in a five- to tenfold decrease in the level of transactivation of the HTLV-II LTR when compared to an equimolar amount of transfected SV-HTLV (Table 1). Transfection of a construct designated SV-HTLV-Cla (Fig. 1B), which is deficient for both *tax* and *rex* because of a frameshift mutation, results in no transactivation above baseline levels, as previously reported (*8*).

To determine whether the mutations affected a trans-acting function of the *rex* gene, a recombinant construct 91023-pX-b, which independently expresses the *rex* gene by means of the eukaryotic expression vector p91023-B, was used (*18*). The sequences surrounding the initiator Met codon for *rex*, GCTGC*ATG*C, match poorly to the consensus initiator sequences identified by Kozak (*19*). By contrast, the sequences surrounding the downstream *tax* gene Met

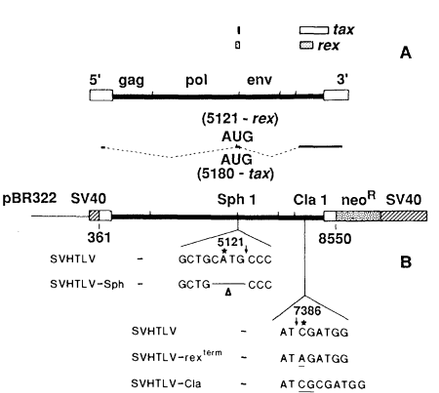

Fig. 1. (**A**) Schematic representation of the HTLV-II genome and coding sequences for nonstructural proteins. The *gag*, *pol*, and *env* coding regions are delineated on the provirus. The boxes above the genome designate coding regions for *tax* and *rex*. The 2.1-kb mRNA species schematically illustrated below the HTLV-II genome contains both *tax* gene sequences encoding $p37^{xII}$, and *rex* gene sequences encoding the other nonstructural protein(s) (*4, 13*). The 5′ leader exon (nt 313 to 449), middle exon (nt 5044 to 5183), and third exon (nt 7214 to 8751) are represented by thick lines. (**B**) Schematic representation of the SV-HTLV expression vector and *rex* gene mutants. The mutations and resultant sequences in SV-HTLV-Sph, SV-HTLV-rex^{term}, and SV-HTLV-Cla are shown below the genome. An arrow designates the Sph I cleavage site at nt 5124 in the SV-HTLV sequence. The Cla I cleavage in SV-HTLV-Cla site is designated by an arrow at nt 7385 in the SV-HTLV sequence.

Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, and Jonsson Comprehensive Cancer Center, Los Angeles, CA 90024.

*Present address, UCSD School of Medicine, Hematology-Oncology, San Diego, CA 92161.
†Present address: Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.