nance for fitness was $h = 0.90$, or nearly complete dominance.

These data demonstrate that the genotype-dependent patterns of biochemical and physiological phenotypes measured under laboratory conditions are best described by dominance of the Lap^{94} allele. We conclude that there is both fitness dominance and phenotypic dominance for a variety of biochemical and physiological phenotypes of the *Lap* locus.

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The *x* Gene Is Essential for HTLV Replication

Abstract. *The human T-cell leukemia viruses (HTLV) are associated with T-cell malignancies in man and will transform normal human T cells in vitro. The mechanism of malignant transformation by HTLV is unknown but appears to be distinct from that of other classes of retroviruses, which induce malignant transformation through viral or cellular oncogenes. Recently a new gene, termed* x, *was identified in HTLV. This gene has been hypothesized to be the transforming gene of HTLV because of its conservation within the HTLV class of retroviruses. By in vitro mutagenesis of the HTLV-II* x *gene, it is now demonstrated that the presence of a functional* x *gene product is necessary for efficient HTLV transcription. Therefore, these studies provide direct evidence for an important function of the* x *gene in HTLV replication. The functional analogies between the* x *gene and transcriptional regulatory genes of some DNA viruses suggest that these viruses share similar mechanisms for cellular transformation.*

IRVIN S. Y. CHEN
DENNIS J. SLAMON
JOSEPH D. ROSENBLATT
NEIL P. SHAH
SHIRLEY G. QUAN
WILLIAM WACHSMAN
Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024

The human T-cell leukemia viruses (HTLV) are associated with specific T-cell malignancies (*1–3*). Human T-cell leukemia virus type I (HTLV-I) is the likely etiologic agent for adult T-cell leukemia (ATL), which is endemic to parts of Japan, the Caribbean, and Africa (*1, 2*). Human T-cell leukemia virus type II (HTLV-II) was associated with a neoplasm in a single patient with a relatively benign T-cell variant of hairy cell leukemia (*4*). Despite the difference in associated diseases, both viruses will infect normal human T cells in vitro, causing their continued proliferation in the absence of exogenous interleukin-2 (*5–8*). The mechanism by which HTLV induces T-cell malignancies in vivo and T-cell transformation in vitro is unknown. Nucleic acid hybridization to cellular DNA (*9–11*) and nucleic acid sequencing (*12–14*) of the complete HTLV-I and HTLV-II proviral genomes have not revealed sequences in HTLV that are related to classical viral oncogenes. Unlike such replication-competent retroviruses as the lymphoid leukosis viruses (*15–17*), Moloney murine leukemia viruses (*18–20*), and mouse mammary tumor viruses (*21*), the HTLV proviral genome does not integrate near specific cellular sequences in tumors (*22*). This finding argues against the theory of transformation by insertional mutagenesis. Therefore, malignant transformation by HTLV appears to involve a novel mechanism.

The genomic structure of HTLV-I and HTLV-II is different from that of other retroviruses. In addition to the *gag*, *pol*, and *env* genes, HTLV has a fourth gene between *env* and the 3′ long terminal repeat (LTR), termed *x* (*12*) [also referred to as *lor* (*14*)]. RNA processing generates a subgenomic messenger RNA (mRNA) (*23*) that encodes a protein of 40 kilodaltons (kD) in HTLV-I–infected cells and 37 kD in HTLV-II–infected cells (*24–26*). The function of these proteins is unknown; however, the conservation of the *x* gene in the genomes of both HTLV-I (*12*) and HTLV-II (*14, 27*) suggests a role for the *x*-encoded protein in cellular transformation. It has been proposed that these proteins have a role in the enhancement of transcription by the HTLV LTR's (*28*), which would be analogous to the immediate early gene functions of the adenoviruses (*29, 30*), the papovaviruses (*31*), and some herpesviruses (*32*). We have used a clone of infectious, proviral HTLV-II DNA (*11, 27, 33*) to study the role of the *x* gene of HTLV in viral replication. The results demonstrate that the *x* gene is essential for high levels of HTLV-II transcription. In the absence of a functional *x* gene, approximately 100-fold lower levels of viral mRNA are transcribed.

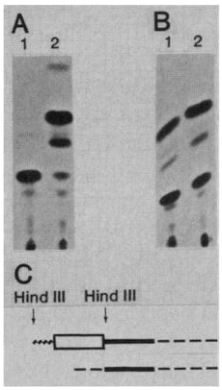
As with T cells, B cells will also support a productive HTLV infection although HTLV does not transform B cells. Some Epstein-Barr virus (EBV)–transformed B-cell lines infected with HTLV-I have been isolated from ATL patients (*34*) and an EBV-transformed B-cell line infected with HTLV-II was obtained from peripheral blood of the patient Mo (*8*). The kinetics of productive infection by HTLV-II in B-cell lines in vitro is similar to in vitro infection of peripheral blood T cells. Therefore, infection of B-cell lines by HTLV provided a model system in which to study HTLV replication. We have previously described a transfection system for HTLV-II in B-cell lines (*27, 33*).

An activity in HTLV-infected cells has been found which enhances the transcription of HTLV LTR's introduced into the cells by DNA transfection (*28*). It was hypothesized that this transcriptional regulatory function was the product of the *x* gene. To examine these findings with respect to HTLV-II, we made a recombinant construct in which the expression of the gene for chloramphenicol acetyltransferase (CAT) (*35*) was dependent on the function of the HTLV-II LTR. We used an HTLV-II

LTR derived from a proviral DNA clone of HTLV-II that had been isolated from HTLV-II–transformed T cells. The clone is infectious in DNA transfection assays (*27, 33*) and results in replication-competent HTLV-II capable of transforming normal human peripheral blood T cells. Thereby we ensured that the LTR was representative of the wild-type HTLV-II genome. The LTR-CAT recombinant constructs were transfected into uninfected and HTLV-II–infected EBV-transformed B-cell lines, termed 729 and 729pH6neo, respectively. Cell line 729pH6neo produces HTLV-II after stable transfection with cloned HTLV-II proviral DNA. Expression of the CAT gene by the LTR's was not detectable in the uninfected cells. In cells infected with HTLV-II, more CAT was expressed under the control of the LTR than by the SV40 early promoter (Fig. 1). Similar results were obtained with a second LTR from another infectious clone of HTLV-II and with a second pair of B cells, one uninfected and the other infected with HTLV-II. Although it is possible that selection for cells competent to transcribe HTLV RNA occurred during the course of infection of cells, it is unlikely to have occurred in the cell line transfected stably by cloned HTLV-II; the only selection was for maintenance of the neo^R marker in the vector. Thus, HTLV-II LTR function is enhanced in the HTLV-II–infected cells. These results are consistent with the demonstration of enhanced function of the HTLV-I LTR in the infected cells of a pair of HTLV-I–infected and –uninfected fibroblast cell lines (*28*). A *trans*-acting viral function in HTLV-II–infected cell lines appears to be necessary for efficient LTR function.

A possible candidate for a virally encoded *trans*-acting protein is the product of the *x* gene, $p37^{xII}$. We constructed mutants of HTLV-II by means of a plasmid (pH6neo) that contains a complete HTLV-II provirus and was previously demonstrated to be infectious (*13, 27, 33*). A unique Cla I site located within the *x* gene (approximately 50 codons from the NH_2 terminus) was cleaved and subjected to Bal 31 exonuclease digestion to generate a series of deletions of varying size within the *x* gene (Fig. 2). The activity of these mutant proviruses was analyzed by DNA transfection into B-cell lines.

The complete wild-type proviral genomes of HTLV subcloned into pSV2neo (*11, 36*) were transfected into a B-cell line, 729-6, and transfected cells were selected for Geneticin (G418) resistance (*33*). About 15 to 20 percent of



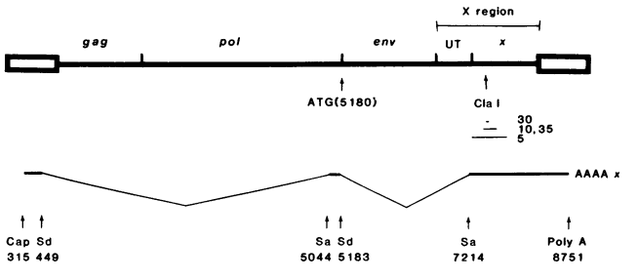
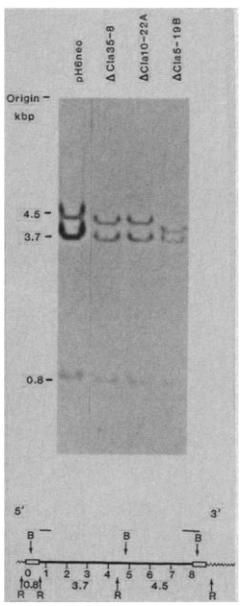


Fig. 1 (left). Expression of chloramphenicol acetyltransferase (CAT) in B-cell lines. Cells (10^7) were transfected with recombinant constructs by standard DEAE dextran procedures (*48*) and harvested 42 hours after transfection. Assays for CAT activity in disrupted cells were as previously described (*35*). Migration of [^{14}C]chloramphenicol in thin-layer chromatography plates is from bottom to top. The acetylated form of chloramphenicol, indicating presence of CAT activity, is the furthest migrating form. (A) pSVCAT, containing the SV40 early promotor, was modified from a previously described construction (*49*). (B) The LTR and flanking cell sequences of an infectious HTLV-II proviral clone (pH6) were isolated by digestion with Hind III (within cell sequences) and Eco RI (nucleotide position 786, 23 nucleotides from the 3′ end of the 5′ LTR) and substituted for the SV40 early promotor of pSVCAT by ligation with Hind III synthetic linkers. (Lane 1) 729-6; (lane 2) 729pH6neo. (C) The LTR CAT and SV CAT constructs. Symbols are wavy line, human DNA sequences; open box, LTR sequences; dotted line, SV40 sequences—early promoter and termination sequences; solid bar, CAT gene. Fig. 2 (right). Schematic representation of the HTLV-II proviral genome and position of the deletions in constructed mutant genomes. The HTLV-II proviral genome is shown schematically at the top with the relative position of the four genes indicated. The X region is as defined by Seiki *et al.* (*12*) and includes an untranslated region (UT) and the *x* gene. The position of the initiation methionine used for both *env* and *x* is indicated. The position of deletions in the X region are indicated beneath the HTLV-II genome as thin solid lines. The number to the right of the deletion designates specific proviral mutants. The structure of the processed HTLV-II mRNA which encodes the *x* gene is shown beneath the HTLV-II genome. The position of the three exons is shown. The numbers beneath the mRNA indicate the nucleotide position of the cap site, splice donors (Sd), splice acceptors (Sa), and polyadenylation sites of the *x* mRNA (*13*). Deletions were generated in the proviral clone of HTLV-II, pH6neo, by cleavage with Cla I followed by Bal 31 exonuclease digestion. Sal I linkers were introduced at the point of deletion to facilitate screening and nucleic acid sequence analysis of mutant proviral DNA.

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                Sa
pH6      CAGCCCATTTCCTAGGATTTGGACAGAGCCTCCTATATGGATACCCCGTCTACGTGTTTG  /270
pACla35  CAGCCCATTTCCTAGGATTTGGACAGAGCCTCCTATATGGATACCCCGTCTACGTGTTTG

pH6      GCGATTGTGTACAGGCCGATTGGTGTCCCGTCTCAGGTGGTCTATGTTCCACCCGCCTAC  /330
pACla35  GCGATTGTGTACAGGCCGATTGGTGTCCCGTCTCAGGTGGTCTATGTTCCACCCGCCTAC

                                                        Cla I
pH6      ATCGACATGCCCTCCTGGCCACCTGTCCAGAGCACCAACTCACCTGGGACCCCATCGATG  /390
                          Sal I   Sal I
pACla35  ATCGACATGCCCTCCTGGCGGTCGACCGGTCGACC·····

pH6      GACGCGTTGTCAGCTCTCCTCTCCAATACCTTATCCCTCGCCTCCCCTCCTTCCCCACCC  /450
pACla35  ·····

pH6      AGAGAACCTCAAGGACCCTCAAGGTCCTTACCCCTCCCACCACTCCTGTCTCCCCCAAGG  /510
pACla35  ·····

pH6      TTCCACCTGCCTTCTTTCAATCAATGCGAAAGCACACCCCCTACCGAAATGGATGCCTGG  /570
pACla35  ·····CGAAAGCACACCCCCTACCGAAATGGATGCCTGG

                Sau 3A
pH6      AACAAACCCTCGGGGATCAGC                                         /630
pACla35  AACAAACCCTCGGGGATCAGC
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Fig. 3 (left). Presence of mutant HTLV-II proviral genomes in 729-6 B cells. Cells were transfected with wild-type HTLV-II, pH6neo, and derivative mutant genomes pΔCla5, pΔCla10, and pΔCla35 by spheroplast fusion as described (*33*). G418-resistant clones of cells were propagated, DNA-extracted, and analyzed by Southern hybridization after digestion with Eco RI. The location of the hybridization probe (thin, straight horizontal line) indicated above the HTLV-II genome and fragments of the cloned wild-type provirus predicted after Eco RI digestion are shown below the autoradiogram. B, Bam HI; R, Eco RI; open box, LTR; wavy line, human DNA sequences. Fig. 4 (right). Nucleic acid sequence of the *x* gene in deletion-mutant clone pΔCla35. The clone was sequenced about the point of deletion in the *x* gene (*50*). The sequence of the wild-type HTLV-II genome (pH6) (*13, 27*) is aligned for comparison. The position of the splice acceptor at the 5′ end of the *x* open reading frame (*23*) is indicated. Only relevant restriction enzyme sites are shown. The nucleotide positions with respect to the entire HTLV-II genome (*13*) are shown to the right of the sequence.

the G418-resistant clones transfected with the wild-type HTLV-II provirus expressed infectious, transforming HTLV-II. In contrast, the transfection of cells by three mutants containing *x* gene deletions of various sizes did not result in stable G418-resistant clones capable of producing infectious transforming HTLV-II. This is despite the fact that more than 90 independent G418-resistant clones of this type were tested for expression of one of the viral core antigens (p19) by indirect immunofluorescence (*8*) and for viral RNA expression by "dot blot" analysis with HTLV-II–specific probes (*33*). These results were confirmed in separate experiments by pooling clones of cells stably transfected with either wild-type provirus or each of the three deletion mutants and testing for production of transforming virus by a cocultivation assay with peripheral blood T cells (*8*). The pooled group of control cells transfected with wild-type HTLV-II was capable of transforming normal human peripheral blood T cells, whereas the pooled cells transfected with mutant HTLV-II did not produce transforming virus. This assay is capable of detecting as few as 50 HTLV-II–infected donor cells. Therefore, deletion mutations in the *x* gene appear to abrogate viral replication.

We further analyzed individual clones of 729 cells stably transfected with mutant HTLV-II to study the genetic basis for the restriction in transcriptional activity. DNA was extracted from several of the cell lines and analyzed by Southern hybridization for the presence of the complete HTLV-II mutant genome. An example of three clones in which the mutant HTLV-II genomes are integrated is shown in Fig. 3. None of these clones expressed detectable viral RNA by dot-blot analysis over a period of 8 months in culture, although the complete mutant genome is present. Furthermore, these clones did not produce transforming virus as assayed by cocultivation with peripheral blood T cells.

The nucleotide sequence surrounding the point of deletion in clone 729ΔCla35-8 was determined (Fig. 4). This clone has a deletion of 187 nucleotides and an insertion of two Sal I synthetic linkers. If $p37^{xII}$ is required for efficient HTLV-II transcription, then superinfection with wild-type HTLV-II should provide wild-type $p37^{xII}$ in *trans* and "rescue" the inactive provirus. We tested this prediction by infecting the 729ΔCla35-8 cells with HTLV-II. Four independent

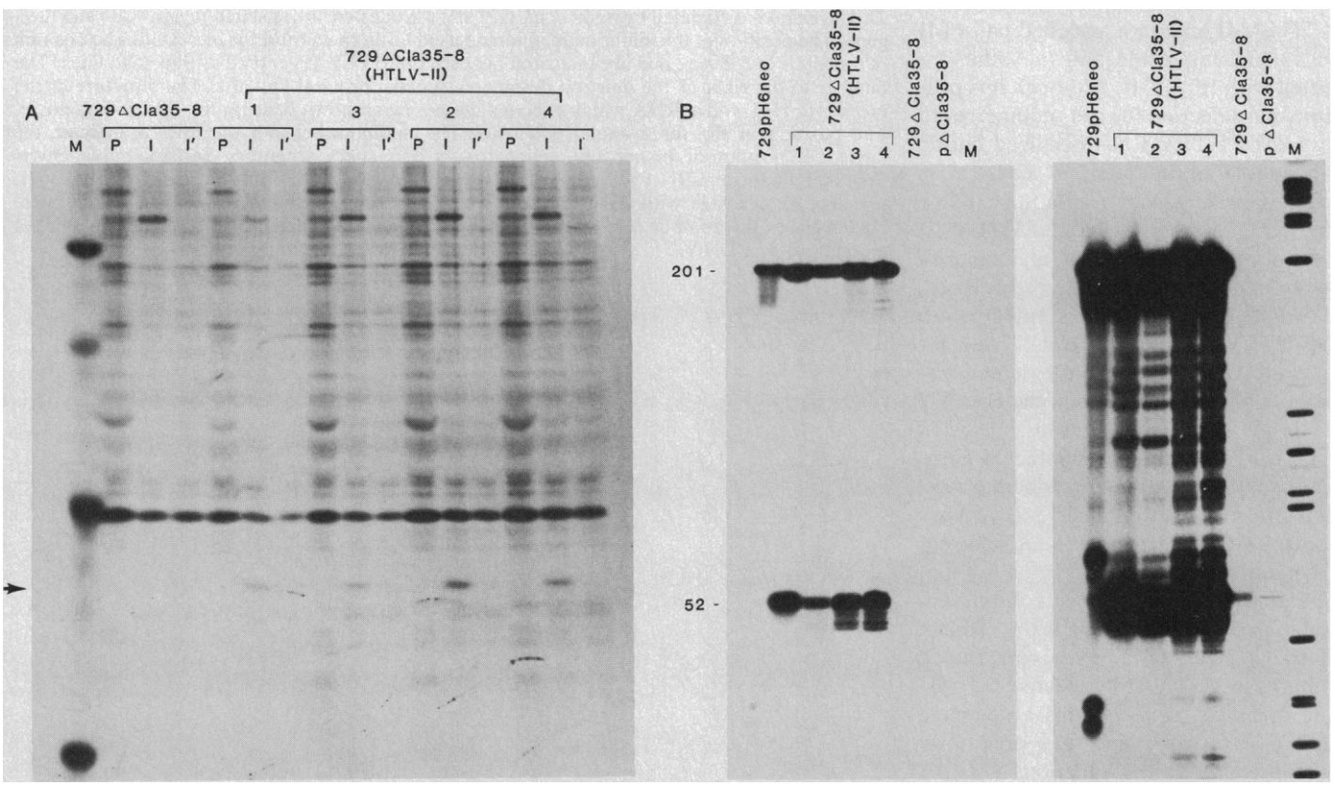

Fig. 5. (A) Radioimmunoprecipitation of ^{35}S methionine-labeled cell lysates with antibodies reactive to HTLV-II $p37^{xII}$ (*24*). Preimmune serum (10 μl), a mixture of two immune antibodies α pXIV-5 and α pXIV-6 (5 μl each) or a mixture of the same antibodies plus the synthetic peptides against which the antibodies are directed (I'), was used to immunoprecipitate $p37^{xII}$ from 10^7 count/min of labeled lysate from 729ΔCla35-8 or from each of four independent HTLV-II–infected lines. Competition with the synthetic peptides was performed as previously described (*24*). P, preimmune sera; I, immune sera; I', immune sera plus synthetic peptides. Arrow indicates the position of $p37^{xII}$. The protein standards (M) are 92.6, 68, 43, and 25.7 kD in size. (B) Expression of mutant HTLV-II RNA in 729ΔCla35-8 cells infected with wild-type HTLV-II. For each infection 5×10^6 729ΔCla35-8 cells were infected with HTLV-II by cocultivation with an equal number of HTLV-II–infected B cells, termed J-WIL. Following 2 days of cocultivation, the J-WIL cells were killed by addition of G418 (1.5 mg/ml) to the culture medium. The infected cells were propagated for an additional 6 weeks, at which time approximately 80 percent of the cells expressed HTLV-II p19 antigen as assayed by indirect immunofluorescence. Total RNA was extracted from four independently infected cell populations and analyzed by S1 nuclease analysis (*51*) in parallel with RNA from the parent cell line, 729ΔCla35-8, and a cell line transfected with the wild-type HTLV-II genome, 729pH6neo. The hybridization probe is a DNA fragment of wild-type HTLV-II from the Cla I site (nucleotide 7385) to the Sau 3A site (nucleotide 7584) (see Fig. 4). The Sau 3A site was labeled with γ ^{32}P ATP and T4 polynucleotide kinase. S1 nuclease assays were performed as previously described with 50 μg of total RNA for all samples, except in the case of 729pH6neo, where 20 μg of RNA was used. The pΔCla35-8 refers to the fragment of the clone from the Sal I site in the linker inserted at the point of deletion in pΔCla35-8 to the Sau 3A site present in the same position as the Sau 3A site of the wild-type genome (see Fig. 4). This fragment was 5′ end-labeled at the Sau 3A site and, therefore, serves as a marker for the distance from the labeled end to the point of deletion. The marker is Sau 3A–digested pBR322 DNA. Sizes are indicated in nucleotides. (Left) Five-hour exposure of gel; (right) 48-hour exposure.

HTLV-II–infected cultures expressed the HTLV-II *x* gene product $p37^{xII}$ (Fig. 5A). As expected, the parent cell line containing the mutant HTLV-II did not express detectable levels of $p37^{xII}$.

We determined whether the resident mutant HTLV-II provirus was transcribed in these infected cells by S1 nuclease analysis. An S1 nuclease hybridization probe that includes the deleted region will distinguish between expression of wild-type and mutant RNA. Wild-type RNA would be expected to protect the entire length of the probe (201 nucleotides), whereas the mutant RNA would protect only a part of the hybridization probe (52 nucleotides). S1 nuclease analysis of RNA from the parent cell line prior to superinfection revealed a low level of HTLV-II ΔCla35 RNA that was detectable only in long autoradiographic exposures (Fig. 5B). This RNA is transcribed beginning at the cap site of the mutant provirus LTR. By contrast, the superinfected cell lines expressed high levels of both wild-type and mutant RNA. The amount of mutant RNA expressed in the superinfected cells is comparable to wild-type RNA and about 100 to 200 times the amount expressed in the parent cells. Only the 201-nucleotide band, representative of wild-type RNA, was detected when RNA from a cell line that had been transfected only with wild-type HTLV-II was analyzed (Fig. 5B, 729pH6neo). To further confirm the "rescue" of the defective genome, we investigated the ability of the mutant genome in the infected cells to be packaged as virions and form unintegrated viral DNA after infection of uninfected 729-6 B cells. Analysis by Southern hybridization of cytoplasmic, linear, unintegrated viral DNA demonstrated the presence of mutant viral DNA, distinguished from wild-type by the presence of a Sal I restriction enzyme site. Similar results were also obtained with the virus that had been rescued from the cell line 729ΔCla10-22A (see Fig. 3).

Thus, the *x* gene of HTLV-II is necessary for efficient transcription of the HTLV genome. A mutant that lacks a functional *x* gene transcribes viral RNA at levels 1/100 to 1/200 as efficiently as the wild-type provirus. These results, considered with the activation of LTR function as assayed by transfection of LTR recombinant constructs into virus-infected cells, indicate that the *x* gene product enhances viral transcription by acting on the initiation of transcription by the HTLV LTR. The recent demonstration of nuclear localization of $p40^{xI}$ is consistent with this function (*26*).

A low level of viral transcription occurs in the absence of *x* gene function. In the mutant provirus of pΔCla35-8 the deletion of sequences and insertion of synthetic oligonucleotide linkers has fortuitously maintained the reading frame (see Fig. 4). Therefore, although unlikely, we cannot exclude the possibility that a weakly functional *x* gene product is present. The deletion results in a loss of a substantial number of amino acids from within the NH_2-terminal region of the protein and adds irrelevant amino acids by the introduction of the two synthetic oligonucleotide linkers. Also, loss of this part of the protein would be expected to be deleterious, because this region is the most highly conserved between HTLV-I and HTLV-II *x* proteins and also has some conserved sequences with bovine leukemia virus (*36*).

The low level of transcription seen in cells transfected with mutant HTLV-II could explain how the wild-type virus replicates. After integration into the genome of the host cell, low levels of *x* mRNA are transcribed, resulting in some $p37^{xII}$. This *x* protein would activate LTR function, in turn producing higher levels of *x* protein to further activate the LTR to transcribe RNA at greater levels. This proposed process is consistent with the kinetics of HTLV infection in B cells (*37*). Unlike a typical retrovirus infection, in which stable RNA expression is dependent solely upon passage of the newly infected cell through the S phase, stable HTLV expression is a much slower process. Multiple cell generations are required to generate high levels of virus expression, consistent with the hypothesis that expression of HTLV occurs via a positive regulatory mechanism.

An *x* gene is also found in bovine leukemia virus (*36*), which shares many molecular, biological, and pathological features with HTLV (*38–40*). The teliologic basis for the evolution of the *x* gene in HTLV/BLV is unknown. Its product may regulate viral expression under some circumstances. A unique mRNA-processing mechanism operates in this retrovirus family whereby the *env* methionine initiation codon and one nucleotide of the next codon are joined to the major open reading frame of the *x* gene to generate the complete coding region (*41, 42*). This processing scheme may be a means of modulating *x* gene expression, thereby allowing further regulation of overall viral expression.

The *x* gene is functionally analogous to *trans*-acting transcriptional regulatory genes of some DNA viruses, the best studied example of which is the EIA gene of adenoviruses. The product of this immediate early gene is required for efficient expression of the other adenovirus genes (*43, 44*); however, a complete replication cycle can occur in the absence of EIA functions, although with delayed kinetics (*45*). The functional analogies between EIA and *x* are relevant to potential mechanisms of HTLV transformation since adenoviruses will transform rodent cells, and EIA has been demonstrated to be capable of transforming cells (*46, 47*). Therefore, the functions of the *x* gene in transcriptional activation are likely to be important in understanding the ability of HTLV to cause T-lymphoid malignancies.

Note added in proof: The low level of viral transcription in the absence of a functional *x* gene is also observed in cell line 729ΔCla5-19B (Figs. 2 and 3) which contains a mutant provirus deleted for more than 500 nucleotides in *x*.

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Steroid-Dependent Survival of Identifiable Neurons in Cultured Ganglia of the Moth *Manduca sexta*

Abstract. *Adult emergence at the end of metamorphosis in the moth* Manduca sexta *is followed by the death of abdominal interneurons and motoneurons. Abdominal ganglia removed from insects before this period of naturally occurring cell death and maintained in vitro showed neuronal death confined to the same cells that normally die in vivo. Addition of physiological levels of the steroid 20-hydroxyecdysone to the culture system prevented the selective death of these motoneurons.*

KELLY L. BENNETT
JAMES W. TRUMAN*
Department of Zoology, University of Washington, Seattle, 98195

*To whom correspondence and requests for reprints should be sent.

Cell death is an important developmental force in determining the final form of the nervous system. Neuronal survival or degeneration is determined by numerous factors, including competition for target sites, the absence of trophic factors, and the presence or absence of various hormones (*1*). In the moth *Manduca sexta* the emergence of the adult at the end of metamorphosis is followed by the rapid loss of about 40 percent of the neurons in the insect's abdominal nervous system (*2*). Many of the cells that die were persistent larval neurons that maintained the behavior of the insect during metamorphosis while the adult nervous system was being formed. This period of postmetamorphic cell death occurs according to a strict temporal program, in which specific cells die in a characteristic sequence (*2*). Studies of intact insects and of isolated abdomens have suggested that the neuronal degeneration was triggered by declining levels of the ecdysteroids, the steroid molting hormones, at the end of metamorphosis (*3*). We used in vitro techniques to show that degeneration results from the direct action of ecdysteroids on the central nervous system (CNS) and also provides insight into the nature of the factors that determine the temporal sequence of cell death.

Segmental abdominal ganglia were taken early in the morning on the last day of adult development and were cultured individually for 24 or 48 hours (*4*). These times were selected because essentially all the cell death in vivo occurs within the first 48 hours after adult emergence (*2*). After the culture period, ganglia were fixed in alcoholic Bouin's fixative, sectioned at 10 μm, and stained with hematoxylin and eosin. Degenerating cells were readily distinguished from healthy cells because the cell bodies of the former were typically condensed, lacked a distinct nucleus, and stained darkly (*2*). The total number of healthy and degenerating cells in the sections from each ganglion was then counted. Interneurons and motoneurons were distinguished on the basis of cell body diameter; diameters of the former ranged from 7 to 15 μm and diameters of the latter ranged from 20 to 40 μm (*5*). Many of the large neurons had characteristic locations in the ganglion, which allowed their unambiguous identification (*6*). These included neurons that survive through adult life and others that normally die after adult emergence.

The tracheal supply was removed along with each ganglion, and an open end was floated on the surface of the medium so that oxygen had direct access to the ganglion. Of those that maintained open tracheal contact with the surface during the culture period, about half showed aberrant histology (such as highly vacuolated cells), which made cell identification and determination of fate impossible. The rest of the cultured ganglia had relatively normal histology and were used in the present analysis.

At the time of explanation, each ganglion contained an average of about 850 neuronal profiles (*2*, *7*). In vivo neuronal counts decreased to 583 ± 20 profiles by 24 hours after ecdysis and to 542 ± 9 at 48 hours (*2*) (mean ± standard error of the mean). After 24 hours in vitro, the number of healthy cells dropped to 691 neuronal profiles and after 48 hours to 618 (Table 1). This reduction in apparent cell number was not an artifact of changes in cell size during the culture period but rather resulted from neuronal death. A moderate number of degenerating interneurons and a few dying motoneurons were evident by 24 hours in vitro (Table 1). After 48 hours in culture, both kinds of neurons showed further increases in the number of degenerating cells.

An important question was whether the neurons that died in vitro were the same cells that die in vivo. After 24 hours in culture, cell death was primarily confined to interneurons in the rostral half of the ganglion. By 48 hours, the

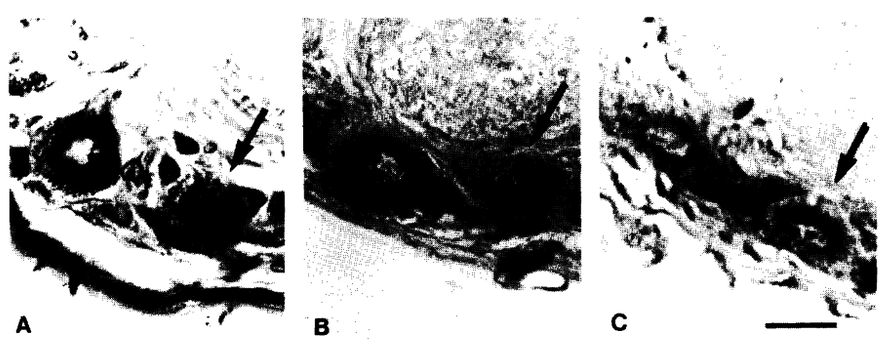

Fig. 1. Histological sections showing the appearance of motoneuron MN-11 (arrow) after exposure of the ganglia to various treatments. (A) By 20 hours after adult ecdysis in vivo, the nucleus has ruptured and condensed as a darkly staining mass in the center of the cell. A neighboring motoneuron (left) is unaffected and will survive through the remainder of adult life. (B) A dying MN-11 motoneuron in a slightly earlier stage of degeneration (the nucleolus is still visible in the middle of the collapsed nucleus) from a ganglion cultured for 24 hours with no hormone. (C) A healthy MN-11 motoneuron with an intact nucleus after 48 hours in vitro with 0.1 μg of 20-HE per milliliter. Scale bar, 20 μm.