during ontogeny of the peripheral nervous system.

Although the developmental potential of premigratory NC cells may be restricted, our results suggest that regional differences in the extracellular matrix encountered during migration in the embryo provide environmental cues responsible for cell line segregation during development of the neural crest. Further investigation is required to elucidate whether the local extracellular matrix instructs developmentally labile NC cells to express a specific phenotype or whether the matrix promotes selective survival and proliferation of phenotypically committed subpopulations. The experimental technique developed in this study offers opportunities for addressing these questions at the molecular level and for characterizing the factors in the extracellular matrix that govern various developmental processes.

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Cellular Transcription Factors and Regulation of IL-2 Receptor Gene Expression by HTLV-I *tax* Gene Product

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Expression of the interleukin-2 receptor (IL-2Rα) gene is activated by the transcriptional activator protein, Tax (previously referred to as the *tat* gene product), encoded by the human T-cell leukemia virus (HTLV-I). Multiple protein binding sites for specific DNA-protein interactions were identified over the upstream IL-2Rα transcriptional regulatory sequences. However, only one region, which includes the sequence motif GGGGAATCTCCC, was required for activation by both the *tax* gene product and mitogenic stimulation. Remarkably, this sequence also bound the nuclear factor NFκB, which is important for induction of κ-immunoglobulin gene expression. A model is presented whereby regulation of cellular gene expression by the HTLV-I *tax* gene product occurs via an indirect mechanism that may involve a post-translational modification of preexistent cellular transcription factors.

THE HUMAN T-CELL LEUKEMIA VIrus (HTLV-I) is the etiological agent of adult T-cell leukemia/lymphoma (ATL) (*1*). The genome of this retrovirus encodes a nuclear transcriptional activator protein, Tax (previously referred to as the *tat* protein), (*2*) that activates gene expression directed by the viral long terminal repeat (LTR) sequences (*3*). Likewise, expression of the *tax* gene activates expression of several cellular genes including the interleukin-2 receptor (IL-2Rα) (*4, 5*). HTLV-I sequences responsive to the *tax* gene product are present on three 21-bp repeats within the LTR (*6, 7*). Recent stud-

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ies suggest that activation of LTR-directed gene expression occurs via an indirect mechanism that may involve activation of a constitutively expressed cellular transcription factor that binds to the 21-bp element (*8*).

The existence of a virus-encoded transcriptional activator protein led to the hypothesis that immortalization of primary lymphocytes following HTLV-I infection results from alteration of cellular gene expression by the *tax* gene product. Consistent with this prediction, studies with transgenic mice confirm that expression of the *tax* gene leads to malignancy (*9*). An attractive target for activation by Tax is the IL-2Rα gene, since both ATL and virus transformed cell lines constitutively express IL-2Rα on their surface (*10*) and growth factor receptors have been implicated as mediators of transformation (*11*). Indeed, recent transient expression studies show that IL-2Rα gene expression is regulated by expression of the HTLV-I *tax* gene product (*4, 5*).

The physiologically active, high-affinity form of the IL-2 receptor is a combination of the 55-kD protein coded by the "TAC" or IL-2Rα gene and the 70-kD protein coded by the IL-2Rβ gene (*12*). Expression of the IL-2Rα gene in response to signals that mimic antigenic stimulation is under tight transcriptional control (*13*), whereas expression of the β gene is thought to be constitutive (*14*). Certain HTLV-I–infected cell lines that express the high-affinity form of the receptor express elevated levels of the IL-2Rα mRNA (*10*).

When nuclear extracts prepared from a wide variety of cell lines were used in gel retardation and methylation interference analyses, multiple protein binding sites were revealed within the IL-2R transcriptional control sequences present between nucleotides −476 to −225 (*15*). To establish the functional significance between host-factor binding and *tax* regulation of IL-2Rα gene expression, we made a series of 5′ deletion mutations (see Fig. 1) within the upstream IL-2Rα regulatory sequence and tested the mutants for activity in the presence of the *tax* protein. So as to measure effects contributed solely from the IL-2Rα 5′ sequences and not the promoter region, we placed the upstream sequences 5′ to an HTLV-I promoter CAT gene cassette sequence lacking an enhancer (Fig. 1). Previous studies showed that the HTLV-I LTR sequences present from −55 to +325 are not in themselves Tax-responsive (7) but respond well to heterologous enhancer signals (7). As shown in Fig. 2, CAT gene expression directed by IL-2Rα sequences −1240 to −225 was activated in the presence of Tax, in accord with previous findings (*4*). S1 nuclease analysis of CAT RNA indicates that activation is at the transcriptional level and that RNA initiates at the correct position within the HTLV-I promoter. On the basis of the 5′ deletion analysis (Fig. 1), a region critical for the Tax response is present between IL-2Rα nucleotides −300 to −257. To confirm this prediction we linked a synthetic oligonucleotide corresponding to nucleotides −327 to −244 5′ to the HTLV-I promoter (plasmid ILR−327/−244) and tested for Tax responsiveness. As shown in Figs. 1 and 2, sequences within this region do confer the Tax-responsive phenotype. Moreover, responsiveness is retained when this region is either inverted in the reverse orientation (plasmid ILR−244/−327) or moved a distance (plasmid ILR−225/−421) from the promoter. Thus, as observed with activation of HTLV-I gene expression, activation of IL-2Rα gene expression by Tax is controlled by an inducible enhancer sequence.

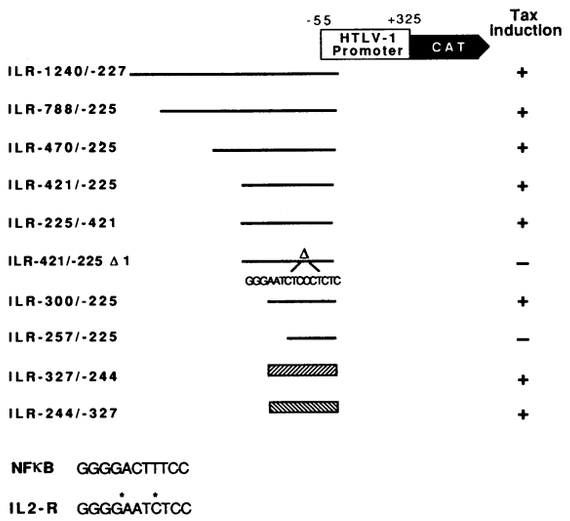

Fig. 1. The IL-2Rα mutations. Sequential 5′ deletions were created with Bal 31 exonuclease (*25*). Plasmids ILR−372/−244 and ILR−244/−327 contain synthetic oligonucleotides that correspond to the indicated nucleotides of IL-2Rα DNA. Site-directed mutagenesis (*26*) was used to create plasmid ILR−421/−225Δ1. All IL-2Rα fragments were cloned according to established protocols (*27*) 5′ to an enhancerless HTLV-I promoter CAT gene cassette (plasmid pC55) (7). Tax induction is scored +/− since, in most instances, the level of gene expression in the absence of Tax was too low to quantitate.

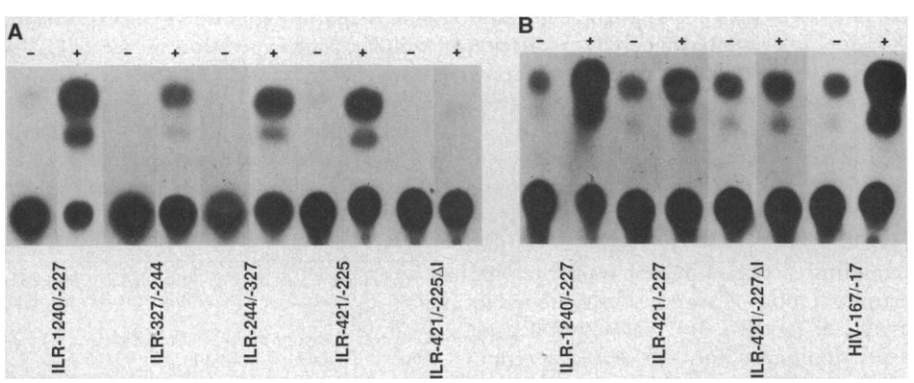

Fig. 2. Activity of IL-2Rα mutations. Representative CAT assays (*28*) obtained from Jurkat cells, transfected with 3 μg of the plasmids indicated, in the presence (+) or absence (−) of Tax (**A**) expressed from plasmid pH *tax*-1 (7), or (**B**) after induction with PHA or PMA (*30*). CAT assays from 30-min reactions are shown.

Analysis of the sequence elements present within the −327 to −244 region did not reveal the existence of consensus sequences for binding of known transcriptional regulatory factors, including AP-1 (*16*), SP-1 (*17*), or factors that bind to the upstream region of cAMP inducible genes (*18*) or the HTLV-I 21-bp element (7). However, the sequence element GGGGAATCTCCC bears striking similarity to the sequence GGGGACTTTCC for the κ-immunoglobulin enhancer binding factor, NFκB (*19, 20*). To examine whether this sequence element was required for Tax regulation, we deleted it from plasmid ILR−421/−225 using site-directed mutagenesis. As shown in Fig. 2, plasmid ILR−421/−225Δ1, which lacks the 15-bp encompassing nucleotides −266 to −252, is no longer Tax-responsive. We conclude that this region is essential for Tax-mediated regulation of gene expression.

The similarity between the sequence present within the IL-2Rα Tax-responsive element and the NFκB binding site suggested a role for NFκB or a related protein in transactivation. To test this possibility, we excised IL-2 receptor sequences from plasmids ILR−441/−225 and ILR−441/−225Δ1. The DNA fragments were then end-labeled

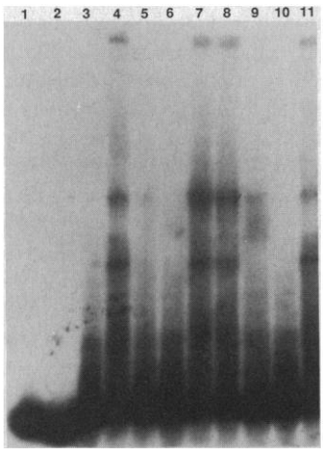

Fig. 3. Binding of wild-type and mutant IL-2Rα DNA by NFκB. End-labeled IL-2Rα DNA obtained from plasmid ILR−421/−225Δ1 (lanes 1 and 2) or ILR−421/−225 (lanes 3 to 11) was incubated in the absence of protein (lanes 1 and 3), or with affinity-purified NFκB protein (lanes 2 and 4 to 11). Competition reactions were performed with 10 ng and 50 ng of oligonucleotides, respectively (*29*), corresponding to an authentic IL-2Rα binding site (lanes 5 and 6), a mutated IL-2Rα binding site (lanes 7 and 8), an authentic NFκB binding site (lanes 9 and 10), or a mutant NFκB binding site (lane 11).

and incubated with affinity-purified NFκB protein (*21*). As shown in Fig. 3, the −421/−225 fragment bound NFκB protein (lane 4), whereas the fragment bearing a deletion of a putative NFκB binding site did not (lane 2). Competition with excess cold oligonucleotide containing the IL-2Rα NFκB binding site reduced binding (lanes 5 and 6), whereas inclusion of excess oligonucleotide bearing two point mutations in this site (lanes 7 and 8) failed to compete with −441/−225 binding. Similar results were observed when we used oligonucleotides bearing authentic and mutant NFκB binding sites, respectively (lanes 9 to 11). On the basis of these competition assays, we conclude that binding of NFκB to the IL-2Rα sequence is specific.

It is established that antigen-specific activation of T cells results in the activation of IL-2 receptor expression; this phenomenon can be mimicked in vitro by mitogenic stimulation (*13*). Similarly, mitogenic stimulation of T and B lymphocytes is thought to activate NFκB through a post-translational modification (*19*). Therefore, the importance of the putative NFκB binding site for mitogenic activation of IL-2Rα gene expression was examined. As shown in Fig. 2B, treatment of Jurkat cells with phorbol 12-myristate 13-acetate (PMA) and a calcium ionophore 24 hours after transfection with plasmid ILR−1240/−235 or plasmid p−167/+80 (*22*) [the latter contains LTR sequences −167 to +80 of the human immunodeficiency virus type 1 (HIV-1) and thus two copies of authentic NFκB binding sites (*23*)], led to the expected marked increase in CAT gene expression. Similarly, activation was evident with plasmid ILR−421/−225. In contrast, no activation was achieved after removal of the NFκB binding sequence. Thus, the sequences required for activation by mitogenic stimuli are identical to those that confer responsiveness to the *tax* protein.

The experiments reported here suggest that activation of IL-2Rα gene expression by the HTLV-I *tax* protein occurs through an interaction with, or activation of, a host transcription factor with properties similar, if not identical, to NFκB. Although several protein binding sites are present within the upstream IL-2Rα regulatory region, only one site is required for transactivation. It is likely that the additional binding proteins play a significant role in regulation of gene expression under different growth conditions.

The mechanism for activation of IL-2Rα expression by Tax remains unclear. Since similar DNA-protein interactions were observed in the presence and absence of Tax, it is unlikely that Tax binds to DNA directly or leads to de novo synthesis of cellular factors that confer activation. More intriguing possibilities are that Tax expression results in the post-translational modification of a constitutively expressed transcription factor from an inactive to an active configuration or that Tax participates directly in the formation of an active transcription complex. Thus, transactivation of IL-2Rα gene expression by Tax would proceed via an indirect, rather than direct, mechanism. Similar mechanisms have been proposed for transactivation of HTLV-I gene expression by Tax (*8*). However, with transactivation of the viral LTR, different DNA sequence elements and most probably a different cellular factor are involved (*8*).

The binding of NFκB to the Tax-responsive IL-2Rα sequences suggests that either NFκB or a protein with a similar recognition sequence is involved in the Tax mediated transactivation pathway. Moreover, the importance of the same sequence for mitogenic inducibility and the observation that NFκB is post-translationally modified by similar stimuli (*19*) suggest that Tax may enter the IL-2Rα regulatory pathway by mimicry of a mitogenic response. We note that activation of IL-2Rα gene expression is evident only in one cell line (Jurkat) of eight tested (*4*), although some other cell lines contain a similar binding activity with the IL-2Rα DNA. A different post-translational modification of the IL-2Rα binding protein may therefore occur in Jurkat cells, or a different cellular protein with the same recognition site may be bound in different cell lines; it has already been reported that a different nuclear factor, H2TF1, binds to a site similar and sometimes identical to the NFκB binding site (*24*). Alternatively, the pathway for activation of this factor by Tax may not operate in other cell types. Further studies to address these possibilities should lead to a better understanding of the role of Tax in cell immortalization.

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30. For PHA and PMA induction, Jurkat cells were treated with 50 ng/ml of TPA and 250 ng/ml of calcium ionophore (Sigma C-9275) 10 hours before the cells were harvested.

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Role of the Gastropod Shell and Operculum in Inhibiting Predation by Fishes

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In contrast to the diets of other cottid fishes and most teleosts, the diet of *Asemichthys taylori* is dominated by gastropod mollusks. Access to this underused prey appears to be made possible by morphological specializations of the neurocranium that allow *Asemichthys* to puncture the shells of its prey during mastication. Unpunched, the shell and operculum act as a barrier to digestion; more than 40% of the unpunched gastropods emerged alive in the feces. *Asemichthys* adjusted its punching behavior in an apparently adaptive way; other prey lacking such barriers to digestion were rarely punched. The ability of some shelled invertebrates to avoid digestion may make them less desirable as prey for many fishes that cannot masticate this kind of prey. The ability of shelled mollusks to survive in the digestive tracts of vertebrates may provide a dispersal mechanism for otherwise sedentary species.

A MAJOR GOAL OF FUNCTIONAL MORphology is the identification of the potential adaptive roles of morphological structures in organisms (*1*). Of equal interest, albeit more difficult in practice, is the identification of limits imposed by morphology on ecology and behavior. Predator-prey interactions involving gastropod mollusks have provided several clear insights into this relation between form and function. Abundant paleontological and neontological evidence documents the strong influence of predation on shell form (*2*). Experiments reveal the role of such gastropod shell features as thickness, spire height, surface ornamentation, and aperture size in thwarting predators such as crabs and fish (*3*). These features are believed to constrain the suite of potential predators on gastropods. Fish species that prey extensively on hard-shelled mollusks are rare among teleosts and usually show structural adaptations for crushing their prey (*4*). In contrast, a wide variety of teleosts with and without crushing morphologies feed successfully on other comparatively hard-shelled organisms such as crabs.

I present evidence that minor modifications of a general body plan can allow a teleostean predator (*Asemichthys taylori*) access to this underutilized prey resource (gastropods). Further, cases of unsuccessful predation by this species highlight the value of a key morphological feature of the prey that may inhibit wider exploitation by fishes.

Asemichthys taylori is a member of the Cottidae, a family of benthic teleostean fishes found primarily in the temperate and boreal Northern Hemisphere. The range of *Asemichthys* extends from southeastern Alaska to Puget Sound, Washington. Cottids are diverse and abundant in intertidal and nearshore subtidal habitats of the northeast Pacific where they are important predators of benthic invertebrates, primarily crustaceans (*5*). The diet of *Asemichthys* differs from that of other cottids (*6*). Gastropods predominate (40% of the diet by mass), with three prosobranch gastropod genera (*Alvinia*, *Margarites*, and *Lacuna*) being the most common (*7*). Predation on a secondary prey, gammarid amphipods (20% by mass), also reflects the strong influence of gastropods in that one gammarid species common in the diet of *Asemichthys* is a Batesian mimic of *Lacuna* and *Margarites* (*8*).

Most teleostean fishes swallow their prey intact with a minimum of mastication (*9*). Most prey in the stomachs of cottid fishes, including *Asemichthys*, are intact. However, most snail shells consumed by this species show a distinct pattern of damage, ranging from a major punch (Fig. 1A) to a row of small holes (Fig. 1B) at a single site on the shell. *Asemichthys* lacks the structural modifications (robust bones and molariform teeth) of the primary jaws or the pharyngeal jaws that allow molluscivorous teleosts to crush their prey. The key morphological innovations producing these holes are found on the vomer, the anteroventral element of the

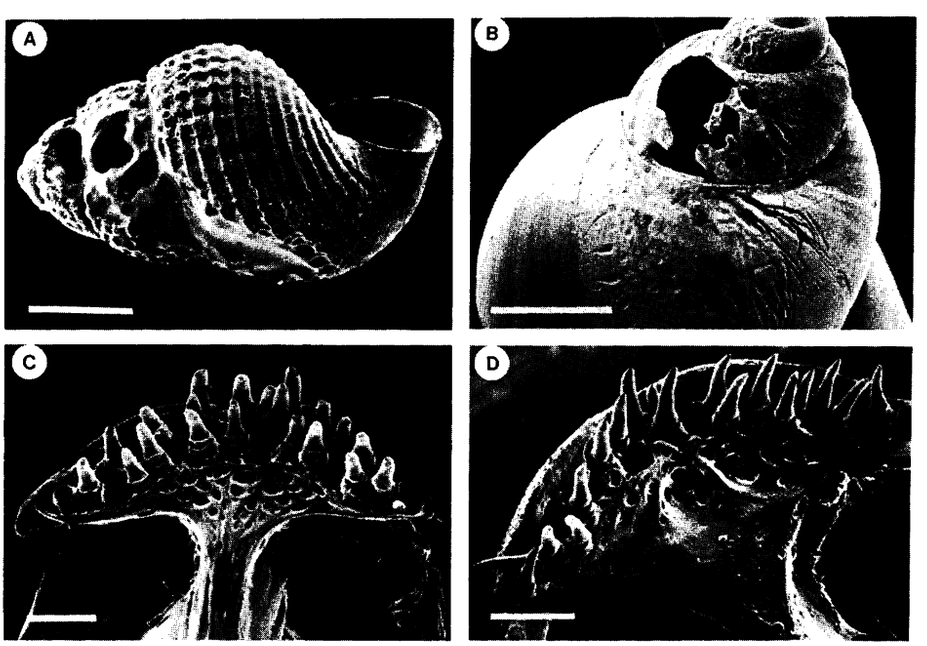

Fig. 1. (**A**) Scanning electron micrograph of an *Alvinia* shell punched by the vomer of *Asemichthys taylori*. Scale bar, 500 μm. (**B**) Scanning electron micrograph of a punched by shell of *Lacuna sp.* Note the scratch marks and the small holes in the shell made by individual teeth. Scale bar, 1000 μm. (**C**) Scanning electron micrograph of the anteroventral region of the neurocranium of a typical cottid, *Artedius harringtoni* (53-mm standard length). Scale bar, 250 μm. (**D**) Scanning electron micrograph of the anteroventral region of the neurocranium of *Asemichthys taylori* (48-mm standard length). Replacement teeth in the process of migrating anteriorly into the main row can be seen on the right side of the vomer. Scale bar, 250 μm.

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