perhaps the TCR α chain, would then elicit an intracellular signal to block further rearrangements of the γ locus. We believe that the transgene-induced inhibition of rearrangements at the endogenous β-chain locus and the γ locus are mediated by the same molecular signal. This could act at the level of the recombinase (or associated factors), or by rendering germline V_γ and V_β segments inaccessible to the recombinase, perhaps by alterations in chromatin configuration or inhibition of transcription at these loci. Whether transgene expression in the TCRE lines causes accelerated developmental activation of the α-chain locus (and hence deletion of δ-chain alleles), and how this might play a role in excluding further TCR gene rearrangements, is unknown. A recent report (*12*) indicates that a TCR β-chain transgene driven by its own promotor and enhancer elements has no discernible effects on γ gene rearrangements. This difference from our observations may reflect the earlier developmental activation of the Ig enhancer in the TCRE mice.

The inherent ability of a functional β-chain complex to block further γ rearrangements would help ensure that each T cell expresses a single idiotypic antigen receptor on its surface. This inhibition of γ rearrangements leads to the prediction that in clonal T cell lines derived from TCRE mice, the γ TCR genes will be in germline configuration and suggests that αβ-bearing T cells can arise without an obligatory developmental stage in which γ genes rearrange. It will be of interest to ascertain whether allelic exclusion also occurs at the TCR δ-chain locus, and to determine directly whether γδ cells can be detected in the skin, thymus, or peripheral lymphoid organs of TCRE animals.

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11 April 1988; accepted 5 July 1988

Tumor Formation Dependent on Proteoglycan Biosynthesis

JEFFREY D. ESKO,* KATHERINE S. ROSTAND, JULIE L. WEINKE

The role proteoglycans play in tumor formation was examined by measuring the tumorigenicity of proteoglycan-deficient Chinese hamster ovary cell mutants in nude mice. When 10^7 cells were injected subcutaneously, mutants with less than about 15% of the wild-type level of proteoglycan synthesis did not produce tumors. Mutants defective in the synthesis of heparan sulfate proteoglycans also did not form tumors, whereas mutants with altered chondroitin sulfate proteoglycans were tumorigenic. Tumors arose from mixtures of wild-type and nontumorigenic mutant cells and contained both cell types, suggesting that wild-type cell proteoglycans enabled mutant cells to survive. The failure of heparan sulfate–deficient mutants to form tumors depended on the ability of the host to mount a B cell–mediated immune reaction.

PROTEOGLYCANS OCCUR ON THE plasma membranes of mammalian cells and in the extracellular matrix. The various classes of proteoglycans are distinguished by differences in core proteins and in the chemical composition of the linear polysaccharide chains (glycosaminoglycans) covalently attached to serine residues in the cores (*1*). The glycosaminoglycans of chondroitin sulfate proteoglycans consist of alternating D-glucuronic acid and *N*-acetyl-D-galactosamine residues, whereas the glycosaminoglycans of heparan sulfate proteoglycans contain alternating residues of D-glucuronic acid or L-iduronic acid and *N*-acetyl-D-glucosamine. Because of the presence of sulfate groups at various positions along the chains and the resulting high density of negative charges, proteoglycans are able to interact electrostatically with numerous soluble ligands and matrix proteins involved in cell adhesion (*2, 3*).

Neoplastic transformation of cells dramatically alters proteoglycan synthesis both in the tumor and in the surrounding tissues (*4*). Although the pattern of these changes varies among tumors of different histological types, in general more chondroitin sulfate proteoglycans and fewer heparan sulfate proteoglycans are present in tumorous tissue than in the corresponding normal tissue (*4*). The heparan sulfate proteoglycans that tumors produce also tend to be poorly sulfated as compared to their normal counterparts (*5*). These alterations in proteoglycan synthesis are thought to stimulate tumorigenic growth by decreasing the adhesion of transformed cells to the extracellular matrix (*6*).

Numerous animal cell mutants have been used to study the function of glycoproteins in tumorigenesis (*7*). A similar approach for studying proteoglycans has not been possi-

J. D. Esko and K. S. Rostand, Department of Biochemistry, Schools of Medicine and Dentistry, and the Comprehensive Cancer Center of Alabama, University of Alabama at Birmingham, Birmingham, AL 35294.
J. L. Weinke, Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294.

*To whom correspondence should be addressed.

ble, because few mutants have been identified (*8*). Recently, we isolated and characterized numerous Chinese hamster ovary cell (CHO) mutants that were defective in proteoglycan synthesis and established the underlying biochemical defects in several of the strains (*9–13*). Mutants defective in the initiation of glycosaminoglycan synthesis do not produce any proteoglycans (*9–11*). Mutants defective specifically in heparan sulfate synthesis (*12*), chondroitin sulfate synthesis, sulfate transport (*10*), and sulfation of heparan sulfate (*13*) also have been found. Because CHO cells form tumors in nude mice, with the mutants we were able to study the role of proteoglycans in tumor formation.

Subcutaneous injection of 10^7 wild-type CHO cells along the lateral abdominal wall of nude (athymic) mice caused tumors in over 85% of the animals (Table 1). Palpable tumors arose within 2 weeks, and after 6 to 10 weeks a typical tumor weighed 1 to 5 g (wet weight). The tumors became vascularized but did not invade surrounding tissues or metastasize to other organs. Cells from a tumor were reestablished in culture (*14*) and, when incubated in defined growth medium, were found to require proline for growth, like the parental CHO-K1 cell line (*15*). Thus, the tumors arose from the injected cells and not from hybrids resulting from the fusion of CHO cells with cells of the host.

We next examined the tumorigenicity of the mutants (see Fig. 1). Injection of 10^7 mutant 745 (xylosyltransferase-deficient) cells did not cause tumors (Table 1). However, injection of 10^7 mutant 745 cells into both sides of an animal or a single injection of 2×10^7 cells caused tumors. Tumor formation by wild-type cells also was dose-dependent, because injection of 10^6 cells caused tumors in only 50% of the animals. Therefore, the effective dose to obtain tumors in 50% of the animals was about 10- to 20-fold greater for mutant 745 cells than for wild-type cells. In our subsequent studies of mutant cells, tumor formation was scored after a single injection of 10^7 cells. Cell dose-response curves for the other mutants have not been analyzed.

Mutant 745 lacks xylosyltransferase (*9*), and, because this enzyme is required for the attachment of xylose to serine residues in the core protein (*1*), the assembly of both heparan sulfate and chondroitin sulfate does not take place. Mutants 761, 618, and 650 also make diminished amounts of heparan sulfate and chondroitin sulfate (Fig. 1), but these mutants lack galactosyltransferase I (*11*), which catalyzes the transfer of galactose to xylose (*1*). Cell hybridization studies showed that these mutants belong to the same genetic complementation group and probably represent different mutant alleles of the galactosyltransferase I gene (*11*). Mutants 761, 618, and 650 made, respectively, 8%, 15%, and 32% of the amount of proteoglycan synthesized by wild-type cells (Table 1). Mutant 761, like mutant 745, did not form tumors, whereas mutants 618 and 650 were tumorigenic. Thus, as little as 15% of the wild-type level of proteoglycan synthesis is sufficient for tumor growth (*16*).

These results suggested that CHO cells require proteoglycans to form tumors in nude mice, but they did not show whether the cells require a specific subclass of proteoglycans. Mutant 803 cells make about 10% as much heparan sulfate proteoglycan and about 50% as much chondroitin sulfate proteoglycan as the parental cell line (*12*). In contrast to mutant 803, mutant 677 does not synthesize any heparan sulfate proteoglycans and makes about three times as much chondroitin sulfate as the wild-type

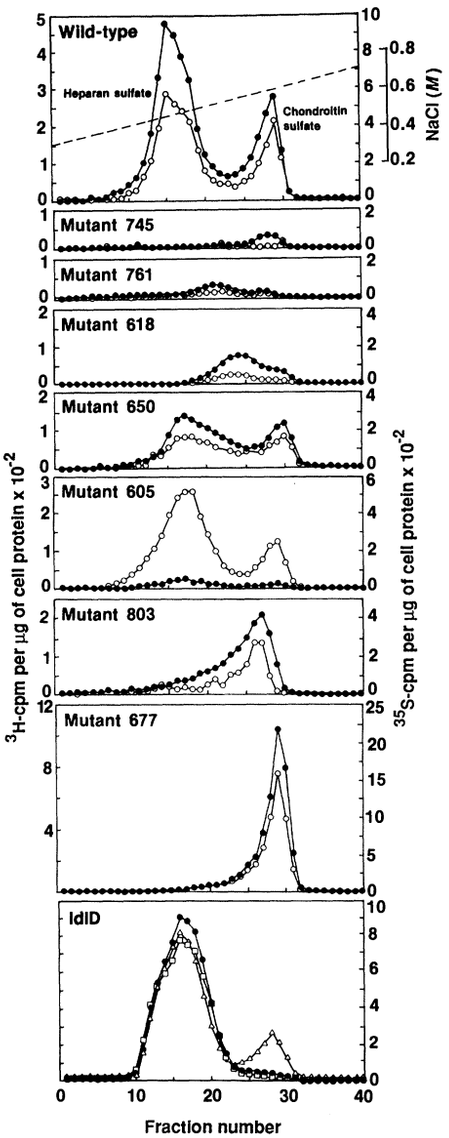

Fig. 1. Anion-exchange HPLC of glycosaminoglycans obtained from mutant and wild-type CHO cells grown in tissue culture. Each mutant was incubated for 3 days in sulfate-free growth medium containing $^{35}SO_4$ (20 µCi/ml) (●) or D-[6-^{3}H]glucosamine (10 µCi/ml) (○), or both, as described (*11*). The labeled glycosaminoglycans were isolated as described (*11*) except that after protease digestion the extract was applied to a 0.5-ml column of DEAE-Sephacel (prepared in a disposable polypropylene pipette tip plugged with glass wool). After the column was washed with 15 ml of 0.3*M* NaCl in 20 m*M* tris-HCl, *p*H 7.4, radioactive glycosaminoglycans were eluted with 2 ml of 1*M* NaCl in 20 m*M* tris-HCl, *p*H 7.4. The samples were precipitated with ethanol: water (4:1, v/v) and resuspended in a small volume of 20 m*M* tris-HCl, *p*H 7.4. A portion was analyzed by anion-exchange HPLC as described (*11*). Radioactivity in each fraction was normalized to cell protein. The broken line in the top panel represents the programmed gradient of sodium chloride. The major peak of radioactivity centered at 0.45*M* NaCl is heparan sulfate, and the smaller peak at 0.59*M* NaCl is chondroitin-4-sulfate (*10*). The IdlD cells were labeled with $^{35}SO_4$ in sulfate-free growth medium (□), in medium supplemented with 10 µ*M* galactose (●), or in medium containing 10 µ*M* galactose and 0.1 m*M* *N*-acetylgalactosamine (△). See Table 1 and (*16*) for a description of the individual mutants.

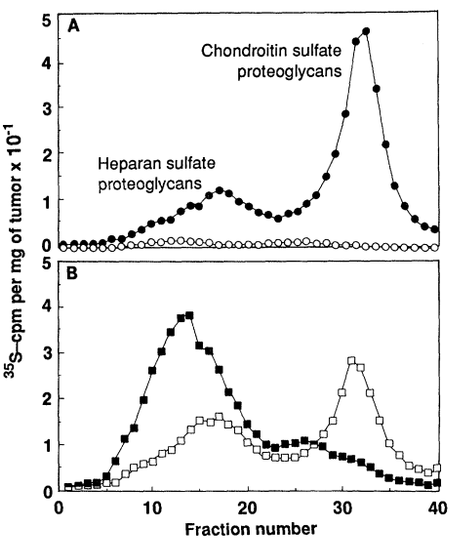

Fig. 2. Tumor proteoglycans. (**A**) Mice were injected bilaterally with either 10^7 wild-type (●) or mutant 745 (○) cells per site. (**B**) Two mice were injected unilaterally with 10^7 IdlD cells. One animal received 0.1% *N*-acetyl-D-galactosamine in its water supply (□), whereas the other animal did not (■). After 4 weeks, the tumors were labeled with 1 mCi of $^{35}SO_4$, and radioactive proteoglycans were extracted as described (*18*). A portion of each preparation was then analyzed by anion-exchange HPLC as originally described for the analysis of CHO cell glycosaminoglycans (*10*) except that the buffers contained 0.2% Zwittergent 3-12 (Calbiochem). Radioactivity in each fraction was normalized to the tumor mass that had been analyzed.

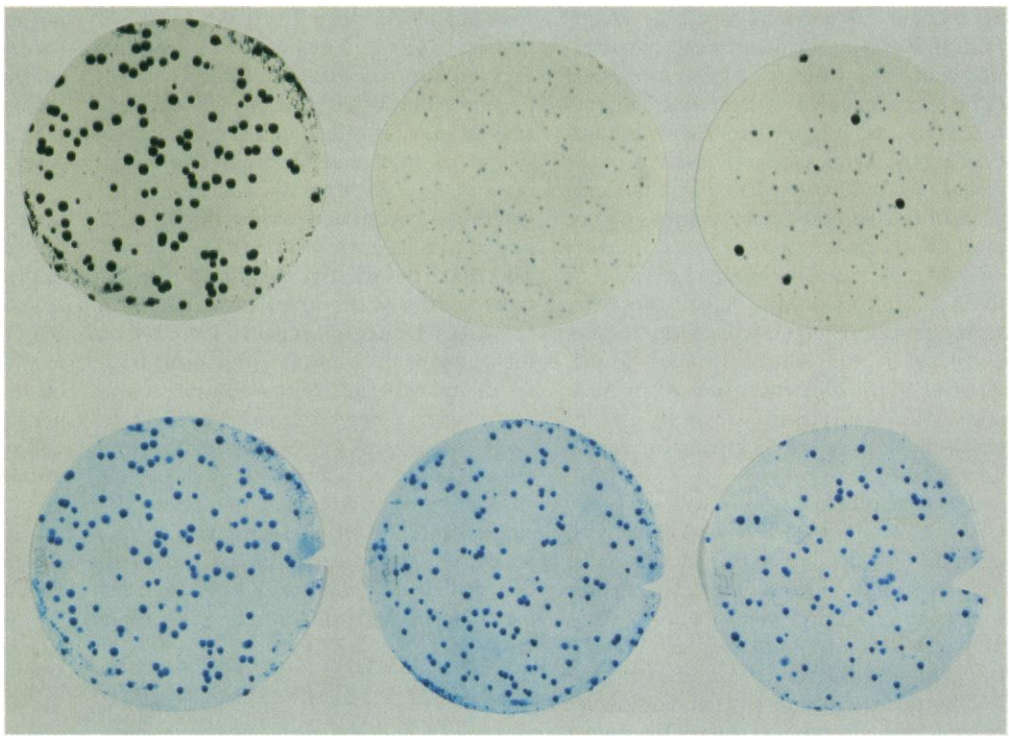

Fig. 3. Tumors derived from mixtures of wild-type and mutant 745 cells contain both cell types. A cell suspension was derived from a tumor that arose from a mixture of 10^6 wild-type cells and 10^7 mutant 745 cells (*14*). The cells were harvested with trypsin, and about 300 cells were added to individual tissue culture dishes. One day later they were overlaid with polyester cloth (*20*). After 10 days at 37°C, the polyester disks were removed and incubated in sulfate-free growth medium. The next day they were incubated for 4 hours in a few milliliters of growth medium containing $^{35}SO_4$. Radioactive proteoglycans were precipitated in situ with trichloroacetic acid, and unincorporated $^{35}SO_4$ was removed on a Buchner funnel under reduced pressure as described (*9*). After exposure to x-ray film, the disks were stained with Coomassie brilliant blue G (*20*). Top row, autoradiograms; bottom row, stained disks. Left, top and bottom, pure wild-type cells; middle, top and bottom, pure mutant 745 cells cloned onto replicas and assayed; right, top and bottom, tumor cells.

cells (Fig. 1). Neither mutant produced tumors, suggesting that CHO cells require at least 15% of the wild-type level of heparan sulfate proteoglycans for tumorigenicity. Because the amount of sulfated proteoglycans in mutant 677 and wild-type cells is comparable (Table 1), the low tumorigenicity of the heparan sulfate–deficient mutants correlates with the lack of heparan sulfate synthesis rather than with the overall extent of proteoglycan synthesis.

To determine if chondroitin sulfate proteoglycans were needed for tumor formation, we examined ldlD cells, which are defective in the de novo synthesis of uridine 5′-diphospho (UDP)–galactose and UDP-*N*-acetylgalactosamine (*17*). When cultured in F12 medium containing 10% dialyzed fetal bovine serum, ldlD cells produced only heparan sulfate (Fig. 1). Because heparan sulfate assembly requires galactose for the formation of the linkage tetrasaccharide (*1*), adequate galactose was derived from degradation of serum glycoproteins, as suggested from studies on glycoprotein oligosaccharide synthesis (*17*). Chondroitin sulfate synthesis did not take place in the presence or absence of added galactose (Fig. 1), suggesting that the cells derived little *N*-acetylgalactosamine from serum glycoproteins. The ldlD cells formed tumors (Table 1), suggesting that chondroitin sulfate proteoglycans were not required for tumorigenicity.

We analyzed the proteoglycan composition of wild-type CHO tumors by injecting $^{35}SO_4$ into each of three tumor-bearing animals (*18*). The tumors contained $(5 \pm 1) \times 10^5$ cpm of ^{35}S-labeled proteoglycans per gram of tumor (wet weight). Analysis of the proteoglycans by anion-exchange high-performance liquid chromatography (HPLC) (Fig. 2) and enzymatic degradation with heparitinase and chondroitinase ABC (*10*) showed that the tumors contained both heparan sulfate and chondroitin sulfate proteoglycans. Because proteoglycans might be contributed by the host through vascular tissue or the blood supply, we examined radioactive proteoglycans in tumors derived from mutant 745, which forms tumors when 10^7 cells are injected into both sides of an animal. Mutant 745 tumors contained 2×10^4 cpm of ^{35}S-labeled proteoglycan per gram of tumor (wet weight), or about

Table 1. Tumor formation by wild-type and proteoglycan-deficient mutant cells. For characterization of the mutants see (*9–12*).

Strain	Biochemical defect	Proteoglycan composition	$^{35}SO_4$ (cpm/μg)*	Frequency of tumors†
Wild-type CHO-K1		Wild-type	8500	19/22
745	Xylosyl-transferase	Proteoglycan-deficient	400	0/28
761	Galactosyl-transferase I	Proteoglycan-deficient	700	1/7
618	Galactosyl-transferase I	Proteoglycan-deficient	1300	3/3
650	Galactosyl-transferase I	Proteoglycan-deficient	2700	4/4
803	?	Heparan sulfate–deficient	3100	0/4
677	?	Heparan sulfate–deficient chondroitin sulfate accumulation	8300	0/7
ldlD (*17*)	UDP-Gal:UDP-Glc 4-epimerase	Chondroitin sulfate–deficient in the absence of *N*-acetyl-galactosamine	6500	3/6

*Mutants were labeled in culture with $^{35}SO_4$, and radioactive glycosaminoglycans were quantified as described in Fig. 1. †Cells were grown in F12 medium supplemented with 10% fetal bovine serum as described (*11*). To measure CHO cell tumorigenicity, we harvested cell monolayers with trypsin and injected 10^7 cells subcutaneously along the lateral abdominal wall of female *nu/nu* Balb/c mice, 7 weeks old (Life Sciences, St. Petersburg, Florida). The cells were injected within 2 hours of trypsin treatment. Animals were maintained in microisolator cages and given unlimited access to sterile chow and water. Tumors arose within 2 to 3 weeks but the animals were observed for up to 3 months.

1/20 of that extracted from wild-type tumors. The extract contained very little material that migrated like proteoglycans extracted from wild-type tumors (Fig. 2A), indicating that more than 95% of the tumor proteoglycans were synthesized by CHO cells.

A tumor derived from ldlD cells contained 4×10^5 cpm of ^{35}S-labeled proteoglycan per gram of tumor (wet weight), and anion-exchange HPLC showed that the proteoglycans contained mostly heparan sulfate chains (Fig. 2B). Chondroitin sulfate proteoglycans did not accumulate as in wild-type cells, suggesting that the circulating level of *N*-acetylgalactosamine in nude mice was low (*19*). Because supplementation of ldlD cells in culture with 0.1 m*M* *N*-acetylgalactosamine restores chondroitin sulfate synthesis (Fig. 1), we investigated whether addition of *N*-acetylgalactosamine to the water supply of the animals would similarly affect the tumor proteoglycan composition. Giving animals *N*-acetylgalactosamine did not affect the efficiency of tumor formation but decreased the amount of ^{35}S-labeled heparan sulfate proteoglycans and increased the amount of ^{35}S-labeled chondroitin sulfate proteoglycans (Fig. 2B). These findings confirm that most of the tumor proteoglycans originated from CHO cells and that the absence of chondroitin sulfate proteoglycans does not affect tumor formation.

In cell culture, about two-thirds of CHO cell proteoglycans are secreted into the medium. Metabolic intermediates inside cells and proteoglycans located on the cell surface constitute the remainder. To test if wild-type heparan sulfate proteoglycans could replace the missing proteoglycan in mutant cells, we injected a mixture of 10^7 mutant 745 cells (xylosyltransferase-deficient) and 10^6 wild-type cells. Tumors arose with high efficiency (3 of 3), suggesting that the mutant did not produce an inhibitor of tumor formation. A cell suspension was derived from one tumor (*14*), and colonies were grown in cell culture and replica-plated to polyester disks (*20*). We determined proteoglycan synthesis in the colonies by measuring the incorporation of $^{35}SO_4$ by autoradiography as described (*20*). About 10% of the colonies took up about ten times as much radioactivity as other colonies on the disk (Fig. 3). The intensity of the autoradiographic image of these colonies was similar to that of wild-type colonies transferred to disks; the signal from the remaining colonies was similar to that observed from mutant 745 colonies transferred to disks. Thus, the tumor contained both mutant and wild-type cells, suggesting that wild-type heparan sulfate proteoglycans enabled the mutant to survive and proliferate. These studies suggested that CHO cells require at least 10% of the normal level of heparan sulfate proteoglycans to form tumors. However, additional experiments showed that as few as 10^3 wild-type cells enabled 10^7 mutant 745 cells to form a tumor, suggesting that the threshold may be less (*21*). The composition and distribution of proteoglycans in mixed tumors have not been evaluated.

We also examined the tumor-forming properties of mutant 745 in various immunologically compromised hosts (*22*). Mutant 745 and wild-type cells formed tumors in irradiated BALB/c mice (4 of 4) and in severe-combined immunodeficient (SCID) mice (3 of 3), which lack both T cells and B cells (*23*). In contrast, mutant 745 cells did not form tumors in homozygous nude/beige mutant mice (0 of 4), which lack T cells and have severalfold less natural killer (NK) cells than nude mice (*24*). Thus, NK cells were not involved in the inhibition of tumor formation. Because nude mice contain B cells but lack mature T cells (*25*), the failure of the mutant to form tumors correlated with a B cell response.

Taken together, these findings suggest that heparan sulfate proteoglycans allow CHO cells to form tumors by interfering with an immune reaction toward the cells. Heparan sulfate proteoglycans on the surface of wild-type cells may act like epiglycanin, a high molecular weight sialoglycoprotein present on mouse mammary carcinoma (Ta3) cells that is thought to mask histocompatibility antigens (*26*). If heparan sulfate proteoglycans shield antigens that can elicit an immune response in nude mice, then the serum of animals injected with mutant cells should contain antibodies to specific antigens present on mutant cells and absent on wild-type cells. Most of the mutants in proteoglycan synthesis have defects that affect chain formation (*9–13*), and underglycosylated core proteins may migrate to the cell surface (*27*). An immune response against underglycosylated proteoglycans, therefore, would preferentially affect mutant cells.

Many tumors are surrounded by a coat of hyaluronic acid (*6*), which can prevent the generation and penetration of cytolytic lymphocytes (*28*). CHO cell tumors may also develop a layer of hyaluronic acid, but this would not explain the failure of the mutants to form tumors because none of the mutations in proteoglycan synthesis should affect hyaluronic acid formation (*1*). Preliminary histological studies indicate that wild-type tumors contain a layer of material near the periphery that stains with alcian blue at *p*H 1, suggesting the presence of sulfated proteoglycan. In an earlier study, it was found that coating tumor cells with heparin altered transplantation and cytotoxicity reactions (*29*), presumably by blocking cell surface antigens. Thus, heparan sulfate proteoglycans also may be deposited in an annulus around the tumor and may modulate the immunoreactivity or accessiblity of the enclosed cells.

Additional studies are needed to establish if heparan sulfate proteoglycans play a role in the proliferation of other types of tumor cells, especially metastatic cells. This is important because the studies of CHO cells describe the tumorigenicity of hamster cells in mice, and the different genetic backgrounds of the cells and the host may affect our results. Since nude mice lack mature T cells, the protection afforded by proteoglycans apparently involves the recognition of T cell–independent antigens. Thus, our findings cannot be generalized to other tumor systems until the tumor-forming properties of proteoglycan-deficient cells is examined in a syngeneic host capable of launching both T cell and B cell reactions.

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16. The possibility that the failure of mutants 745 and 761 to form tumors is due to a secondary mutation unrelated to the defect in proteoglycan synthesis seems unlikely, because the same secondary mutation would have had to occur in the different strains. We also found that mutant 605 formed tumors efficiently (4 of 4). Mutant 605 is defective in the transport of inorganic sulfate from the medium (Fig. 1), but it synthesizes fully sulfated proteoglycans using inorganic sulfate generated from the catabolism of sulfur-containing amino acids (*10*).
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18. One millicurie of carrier-free $Na^{35}SO_4$ (Amersham, Arlington, IL) was diluted to 0.5 ml with phosphate-buffered saline, filter-sterilized, and injected intraperitoneally. Eighteen hours later, the tumors were excised, weighed, and extracted with 4*M* guanidine hydrochloride containing leupeptin (0.5 μg/ml), pepstatin A (1 μg/ml), 2 m*M* phenylmethylsulfonylfluoride, 20 m*M* *N*-ethylmaleimide, 10 m*M* EDTA, and 50 m*M* sodium acetate, *p*H 6.0 [V. C. Hascall and J. H. Kimura, *Methods Enzymol.* **82**, 769 (1982)]. The extracts were filtered through gauze, and the filtrate was dialyzed against 0.3*M* NaCl, 4*M* urea, and 50 m*M* sodium acetate buffer, *p*H 6, containing protease inhibitors. The sample was next passed over a small column (0.5 ml) of DEAE-Sephacel (Pharmacia), and non-proteoglycan contaminants were washed from the column with 15 ml of solution. We recovered the proteoglycans by increasing the salt concentration to 1*M*. A portion of this material was counted by liquid scintillation spectrometry.
19. The material in the tumor that migrated in the region of chondroitin sulfate has not been identified (Fig. 2B), but more highly sulfated forms of heparan sulfate proteoglycan elute in this position.
20. For animal cell replica plating, see J. D. Esko, *Methods Enzymol.* **129**, 237 (1986); *Methods Cell Biol.*, in press.
21. To test how many wild-type cells might be needed to satisfy the proteoglycan requirement of the mutant, we examined the cell composition of tumors derived from mixtures of 10^7 mutant 745 cells and 10, 10^2, 10^3, 10^4, 10^5, or 10^6 OUA-1 cells, a CHO mutant resistant to 1 m*M* ouabain. OUA-1 synthesizes the same proteoglycans as those produced by CHO-K1 and forms tumors efficiently (14 of 15). Tumors arose from mixtures containing $\geq 10^3$ OUA-1 cells, and incubation of cell suspensions from each mixed tumor in growth medium with and without ouabain showed that the ratio of the two cell types in each tumor was within a factor of 2 to 8 of their ratio in the original mixture injected into the animals.
22. BALB/c mice were γ-irradiated with 650 rads from a ^{60}Co source. One day later, the animals were injected with cells. Nude/beige mice contain about 1/7 as many NK cells as homozygous nude mice (*24*). SCID mice, provided by J. McGhee (University of Alabama at Birmingham), were maintained under sterile conditions in a Trexler isolator. CHO cells formed tumors rapidly in SCID mice and metastasized to other sites.
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30. We thank M. Cooper, M. Höök, A. Kraft, A. Lobuglio, S. Hajduk, K. Bame, and R. Montgomery for discussions. This work was supported by NIH grant GM33063 (J.D.E.), a Basil O'Connor starter grant from the March of Dimes Birth Defects Foundation, and research development program awards from the Comprehensive Cancer Center and the Cystic Fibrosis Center at the University of Alabama at Birmingham.

15 March 1988; accepted 5 July 1988

A Novel Target for Antidiuretic Hormone in Insects

JEFFREY H. SPRING, ANNE M. MORGAN, SHELIA R. HAZELTON

Diuresis in insects is controlled by two antagonistic hormone groups: diuretic hormones, which promote water loss, and antidiuretic hormones, which inhibit it. All known antidiuretic factors act solely to promote fluid reabsorption by the hindgut and do not affect secretion by the Malpighian tubules. In the house cricket, *Acheta domesticus*, an antidiuretic hormone was found that inhibits fluid secretion by the Malpighian tubules but has no effect on the hindgut. Correlations were found between the density of neurosecretory granules and the presence of antidiuretic hormone in the corpora cardiaca, suggesting that the hormone is released from specific axons. Its release is triggered by dehydration; the hormone is detectable in the hemolymph of water-deprived crickets. These results imply that an unusual mechanism regulates water balance in these insects.

THE CURRENT MODEL FOR THE CONTROL of diuresis in non–blood-feeding insects is based on the presence of two antagonistic hormone groups. Diuretic hormones (DHs) stimulate fluid secretion by the Malpighian tubules and inhibit reabsorption by the rectum (*1*), whereas an antidiuretic hormone (ADH) enhances fluid resorption by the hindgut (*2*, *3*). It was reported that ADH could also inhibit fluid secretion (*4*); however, those results subsequently could not be duplicated (*3*, *5*, *6*). On the basis of in vitro measurements of DH degradation rates, and in the absence of data confirming the action of ADH on tubules, it was proposed that tubular secretion is reduced solely by enzymatic destruction of DH and by a reduction in DH release (*2*, *7*).

We have reported that diuresis by the Malpighian tubules of the house cricket, *Acheta domesticus*, was stimulated by homogenates of the primary neurohemal organs [corpora cardiaca (CC)] as well as homogenates of several other ganglia (*8*). To distinguish between the physiological and pharmacological effects of such treatments, we also performed bioassays on extracts of the hemolymph to determine which factors were present under various conditions of water stress. We determined that hemolymph could either stimulate or inhibit fluid secretion by the tubules in vitro, depending on the hydration state of the donor animal.

To produce different hydration states, crickets were subjected to one of three preliminary treatments. One group of insects was freely provided with food and water for 48 hours (control). A second group had access to food and water for 24 hours, after

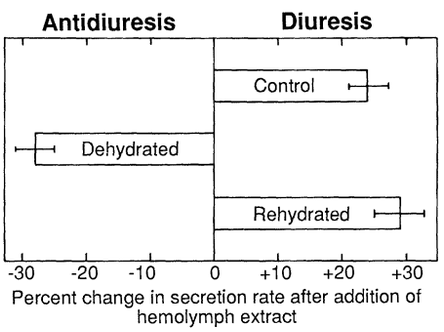

Fig. 1. Histogram of the maximum change in secretion rate by the Malpighian tubules in vitro (mean ± SE) in response to methanolic extracts of hemolymph. Hemolymph from crickets allowed free access to food and water (control, $n = 9$) acts as a diuretic, promoting fluid secretion by the tubules. When control crickets were held at 0% RH for 24 hours (dehydrated, $n = 12$) the hemolymph acted as an antidiuretic, inhibiting fluid secretion. Dehydrated crickets allowed access to water for 1 to 2 hours (rehydrated, $n = 8$) produced hemolymph which also acted as a diuretic, with a slightly greater effect than observed in the controls (not significant at $P = 0.05$, Student t test).

Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504.