REFERENCES AND NOTES

1. S. M. Awramik and L. Margulis, in *Stromatolites*, M. R. Walter, Ed. (Elsevier, Amsterdam, 1976), p. 1.
2. B. W. Logan, R. Rezak, R. N. Ginsburg, *J. Geol.* **72**, 68 (1964); C. D. Gebelein, *J. Sediment. Petrol.* **39**, 49 (1969); R. J. Horodyski, *J. Paleontol.* **51**, 661 (1977).
3. Tropisms or tropic responses are found in organisms that are attached to a substratum and are incapable of taxis [M. J. Carlile, in *Primitive Sensory and Communication Systems*, M. J. Carlile, Ed. (Academic Press, New York, 1975), pp. 1–28]. Although motility and thus phototaxis is prevalent in filaments building stromatolites, attached cyanobacteria like the coccoid *Entophysalis major* can construct stromatolites [S. Golubic, in *Biomineralization and Biological Metal Accumulation*, P. Westbroek and E. W. deJong, Eds. (Reidel, Dordrecht, 1983), pp. 313–326]. The attached photosynthetic microbes and nonmotile cyanobacterial filaments that occur must be positively phototropic.
4. Although cyanobacteria are the most common constructors, other microbes are known to participate. Red algae [P. Hommeril and M. Rioult, *Mar. Geol.* **3**, 131 (1965)], diatoms [J. Bauld, in *Microbial Mats: Stromatolites*, Y. Cohen, R. W. Castenholz, H. O. Halvorson, Eds. (Liss, New York, 1984), pp. 39–58], flexibacteria (*21*), and apparently even fungi [M. Kretzschmar, *Facies* **7**, 237 (1982)] can produce stromatolite-like structures.
5. S. C. Nordeng, *Proceeding of the Fifth Inst. Lake Superior Geology Annual Meeting* (University of Minnesota, Minneapolis, 1959), p. 9; *Soc. Econ. Palaeontol. Min. Spec. Publ.* **10**, 131 (1963); A. G. Vologdin, *Priroda* **11**, 102 (1961); *Akad. Nauk SSSR Dokl.* **151**, 683 (1963).
6. V. P. Maslov, *Probl. Palaeontol. (Moscow Univ.)* **4**, 324 (1938); R. Rezak, *U.S. Geol. Surv. Prof. Pap.* **294-D**, 127 (1957).
7. P. Hoffman, *Science* **157**, 1043 (1967).
8. C. D. Gebelein in (*2*); H. J. Hofmann, *Earth-Sci. Rev.* **7**, 339 (1973); B. W. Logan, P. Hoffman, C. D. Gebelein, *Mem. Am. Assoc. Petrol. Geol.* **22**, 140 (1974).
9. P. E. Playford, *W. Aust. Geol. Surv. Annu. Rep. 73* (1980). In some cases, oversteepening away from current has been observed [B. W. Logan *et al.*, in (*8*)].
10. P. Hoffman, in *Stromatolites*, M. R. Walter, Ed. (Elsevier, Amsterdam, 1976), pp. 261–271.
11. J. R. McAllister and R. J. Ross, *Bull. Am. Assoc. Petrol. Geol.* **62**, 541 (1978).
12. J. P. Vanyo and S. M. Awramik, *Geophys. Res. Lett.* **9**, 1125 (1982); *Precambrian Res.* **29**, 121 (1985).
13. W. H. Munk, in *The Earth-Moon System*, B. G. Marsden and A. G. W. Cameron, Eds. (Plenum, New York, 1966), pp. 52–69.
14. J. W. Wells, *Nature (London)* **197**, 948 (1963); G. Pannella, C. MacClintock, M. N. Thompson, *Science* **162**, 792 (1968).
15. Declination of the inclined subtidal stromatolites was not measured in situ but from photographs. Observations and photography (March 1985) were made while facing due east. The rays of specular sunlight in the slightly turbid water provide documentation (Fig. 2A).
16. P. E. Playford and A. E. Cockbain, in *Stromatolites*, M. R. Walter, Ed. (Elsevier, Amsterdam, 1976), pp. 389–411.
17. It is curious that both in Hamelin Pool and in Yellowstone, previous investigators looked for, but did not find, evidence of heliotropically inclined recent stromatolites [see (*16*) and Walter *et al.*, in (*21*)]. We cannot explain this discrepancy. Detection of north-south tilt is enhanced when the observer looks due east or west at about ground level especially in conjunction with precise vertical and north-south reference instruments. Southerly inclined, desiccated, *Lyngbya*-dominated tufts were found (July 1985) in Bonaire, Netherland Antilles, employing this method.
18. Dominant diatoms are *Mastogloia* spp., *Nitzschia* sp., *Amphora* spp., *Diploneis* sp., *Anomoeoneis* sp., *Navicula* sp., and *Fragilaria* sp. [identifications by R. W. Holmes].
19. P. E. Playford, in (*9*).
20. J. P. Vanyo, R. A. Hutchinson, S. M. Awramik, *Eos*, in press. Observations were made in September 1984 and March 1985. March zenith angles tended to be greater, reflecting the winter inclination of the sun.
21. D. Ward (personal communication) confirms the presence of these microorganisms as initially reported by M. R. Walter, J. Bauld, T. D. Brock, in *Stromatolites*, M. R. Walter, Ed. (Elsevier, Amsterdam, 1976), pp. 273–310.
22. We thank D. Pierce, R. W. Holmes, R. A. Hutchinson, D. Ward, and P. E. Playford for their expertise and assistance. Appreciation to the Department of Conservation and Land Management (on the recommendation of the Department of Mines), Western Australia, and the U.S. National Park Service for permission to conduct research in Hamelin Pool and Yellowstone National Park. Thanks also to WQED Pittsburgh "Planet Earth" project for the opportunity to visit Hamelin Pool. Supported by grant EAR83-03754 from the National Science Foundation. Contribution 147 of the Preston Cloud Research Laboratory.

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Human Melanoma Proteoglycan: Expression in Hybrids Controlled by Intrinsic and Extrinsic Signals

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Human malignant melanoma cells express specific chondroitin sulfate proteoglycans (mel-CSPG) on the surface, both in vivo and in vitro. Melanocytes in normal skin show no detectable mel-CSPG but can be induced to express the antigen when cultured in the presence of cholera toxin and the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate. Most other cell types do not express mel-CSPG either in vivo or in vitro. A study was designed to examine regulatory signals controlling mel-CSPG expression. The gene encoding mel-CSPG was mapped to human chromosome 15, and this chromosome was introduced into rodent cells derived from distinct differentiation lineages. Three types of mel-CSPG–expressing hybrids were found: (i) hybrids derived from human melanomas; (ii) hybrids derived from human cells that do not express mel-CSPG; and (iii) hybrids derived from human cells expressing mel-CSPG that are antigen-negative but that are induced to express mel-CSPG when cultured on extracellular matrix instead of plastic surfaces. Thus, mel-CSPG expression can be controlled both through intrinsic signals, provided by the differentiation program of the rodent fusion partner, and through extrinsic signals, provided by specific cell-matrix interactions.

MONOCLONAL ANTIBODIES HAVE been used to identify a large number of surface antigens on human melanoma cells (*1*). One of these antigens, a high molecular weight glycoconjugate, shows a highly restricted distribution on cultured cells and in tissues (*2–6*). Biochemical analysis has shown that the antigen is a chondroitin sulfate proteoglycan (mel-CSPG), consisting of a 250-kD core glycoprotein and a number of characteristic glycosaminoglycan side chains (*7*). The molecular basis for the expression of this proteoglycan in melanomas is not known. Since normal skin melanocytes do not express detectable levels of mel-CSPG in vivo, antigen expression in melanomas may be part of their transformed phenotype. However, nevi, which are benign disorders of melanocytic cells, and skin melanocytes cultured in vitro in the presence of cholera toxin and the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate also express mel-CSPG (*3–5*). Hence, antigen expression is not restricted to malignant tumors. The extracellular and cell membrane localization of proteoglycans in other cell types suggests that these molecules have a role in cell-matrix interactions and tissue organization. Because mel-CSPG may have a similar role in cells expressing this antigen (*7*), we investigated the relation between mel-CSPG expression and cell lineage and cell-matrix interaction through analysis of rodent-human somatic cell hybrids.

Rodent-human hybrid clones were generated by fusing different types of human cells (melanoma, neuroblastoma, lymphocytes, and kidney epithelial cells) with rodent cells (L-cell and A9 mouse fibroblasts, N4TG-1 and NS20TG11E mouse neuroblastomas, RAG mouse renal cancer cells, and YH21 Chinese hamster ovary fibroblasts). The human chromosomes retained in the hybrid clones were determined by karyotype analysis and typing for human isozyme and cell surface markers (*8, 9*). Expression of mel-CSPG in hybrid cells was tested with monoclonal antibodies in mixed hemadsorption (MHA) assays and radioimmunoprecipitation tests. Six monoclonal antibodies recog-

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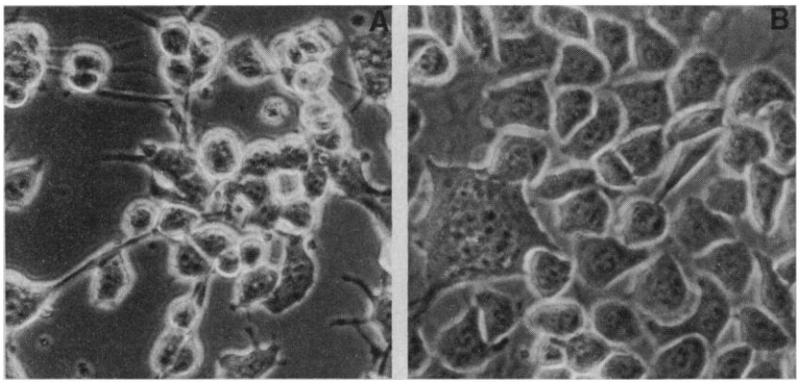

Fig. 1. Morphology of NBE-K1 mouse-human neuroblastoma hybrid cells grown on plastic dishes (Nunc, Roskilde, Denmark) or on plastic dishes coated with extracellular matrix derived from bovine corneal endothelial cells (International Bio-Technologies, Jerusalem, Israel). Cells maintained in plastic flasks were seeded at identical cell densities into (A) a plastic dish or (B) an ECM-coated dish and phase-contrast photomicrographs were taken after 10 days of culture. Cells grown on plastic are round, extend processes, do not form confluent sheaths of cells, are loosely substrate-adherent and are rapidly detached by incubation in phosphate-buffered saline (PBS); cells plated on ECM-coated substrates spread out and adhere within 24 hours and, when confluent, form a continuous sheath of cells with a cobblestone pattern. They remain substrate-adherent when incubated in PBS.

nizing mel-CSPG were used in this study; three antibodies (designated B5, 9.2.27, and 222.28) were derived against human melanomas (*2–4*), and three (designated A050, A092, and A0122) were derived against human astrocytomas (*10*). In initial experiments we used sequential immunoprecipitation assays with extracts of radiolabeled melanoma cells to establish that all six antibodies are reactive with the same cell surface components. We further defined the relation between the epitopes detected by antibody competition assays (*11*). The results suggested that at least four epitopes of mel-CSPG could be distinguished with these antibodies. The 9.2.27 epitope had been shown earlier to be present on the 250-kD core protein of mel-CSPG (*7*), and we have determined by immunoblotting procedures that antibody B5 also reacts with the core protein and not with the glycosaminoglycan side chains.

A series of 26 hybrid clones was typed by MHA assay with monoclonal antibodies B5, 9.2.27, 225.28, and A0122 to the four distinct mel-CSPG epitopes. A comparison between the serological typing results and the distribution of individual human chromosomes among these hybrid clones permitted the assignment of gene loci controlling the expression of B5, 9.2.27, 225.28, and A0122 epitopes to human chromosome 15 (Table 1). Since antibodies B5 and 9.2.27 detect the core glycoprotein of mel-CSPG, we assign the gene coding for this molecule to human chromosome 15. Furthermore, since our discordancy analysis shows that the A0122 and 225.28 epitopes are also controlled by this chromosome, we suggest that these epitopes are on the core protein; alternatively, they may be on the glycosaminoglycan side chains of mel-CSPG, and expression of these side chains is also determined by chromosome 15.

Additional hybrid clones containing human chromosome 15 were derived by fusing human cells that express mel-CSPG or human cells that do not express mel-CSPG (mel-CSPG$^-$) with different rodent cell types. Serological typing of these hybrid clones has permitted the analysis of regulatory mechanisms affecting mel-CSPG expression. Six of 22 hybrid clones derived from human melanoma retained human chromosome 15, and all six clones (CE12, AM1, AM3.8, AM18.6, LM13.20, and LM39) expressed mel-CSPG (reciprocal titers in MHA assays, 1×10^5 to 5×10^5). The melanoma hybrids lacking human chromosome 15 were mel-CSPG$^-$, consistent with our chromosomal assignment. Induction of mel-CSPG expression was found in certain hybrids derived from human mel-CSPG$^-$ cells. Four hybrid clones constructed with mel-CSPG$^-$ human kidney epithelial cells and mouse A9 fibroblasts (clones ANK4/4, ANK4/14, ANK4/20, and ANK4/23) and one clone constructed with mel-CSPG$^-$ human lymphocytes and A9 (clone A9/DXB-6) expressed mel-CSPG as determined by MHA assays with monoclonal antibodies A0122 and B5. In contrast, six hybrid clones constructed with human kidney epithelial cells and RAG mouse renal cancer cells (clones RC1, RC2, RC3, RC4, RC5, and RC7), which had also retained a normal human chromosome 15, did not express mel-CSPG. These results show that mel-CSPG is induced by fusing nonexpressor human cells with rodent cells derived from specific cell lineages; mouse fibroblasts induce mel-CSPG expression, but mouse renal cancer cells do not.

We also examined the influence of cell-matrix interactions on mel-CSPG expression in hybrids containing human chromosome 15. A number of hybrid clones derived from fusions between human and mouse neuroblastoma cells (designated as NBE series) showed concomitant changes in cell morphology, substrate adhesiveness, and mel-CSPG expression when grown on substrates coated with extracellular matrix (ECM) instead of plastic surfaces. Figure 1 illustrates the change in cell morphology and substrate adhesion observed in a typical NBE neuroblastoma hybrid clone after it is transferred from a plastic dish to an ECM-coated dish. Figure 2 illustrates the accompanying

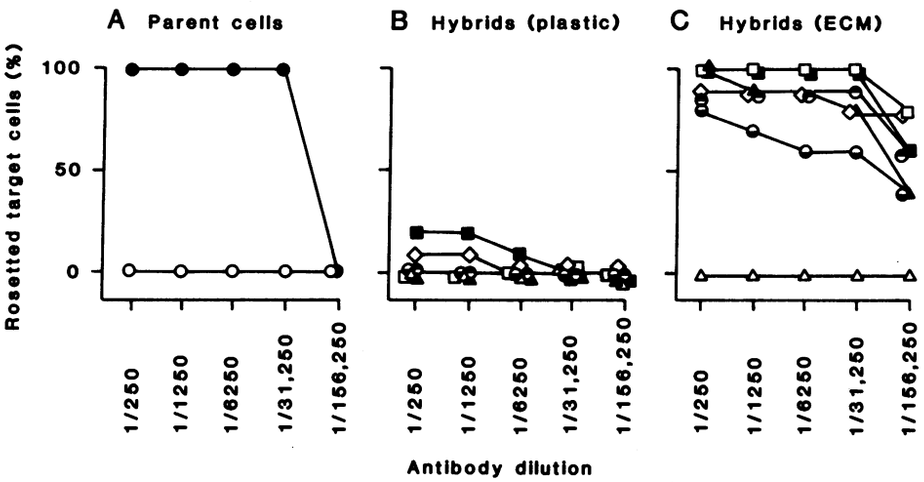

Fig. 2. Surface expression of mel-CSPG on cultured cells tested with serial dilutions of monoclonal antibody A0122 in MHA assays (*10*). (A) Human neuroblastoma cell line BE(2)-C (●) and mouse neuroblastoma cell line NS20TG11E (○). (B) NBE hybrid clones C2 (■), D4 (□), E1 (▲), G1 (△), H1 (◓), K1 (◒), and N1 (◇) grown on plastic surfaces. (C) NBE hybrid clones (indicated as above) grown on ECM-coated Terasaki plates for 48 hours prior to MHA assays. Hybrid clones J2 and M1 (not shown) gave identical patterns of reactivity to N1.

change in mel-CSPG expression as determined by MHA assays. NBE cells express little or no mel-CSPG when grown on plastic surfaces; however, within 24 hours after transfer to ECM-coated dishes, all eight NBE hybrid clones containing human chromosome 15 become strong mel-CSP expressors. As expected, NBE-G1 cells, which do not contain human chromosome 15, remain negative for mel-CSPG expression when grown on ECM although they show changes in cell morphology similar to those that accompany mel-CSPG expression. No changes in cell morphology or mel-CSPG expression were seen in the melanoma-derived hybrids when they were transferred from plastic to ECM-coated dishes. Figure 3 shows that mel-CSPG can be immunoprecipitated from ECM-induced NBE-K1 hybrid cultures but not from non-induced cultures; therefore, the increase in surface expression of mel-CSPG in hybrids grown on ECM reflects an increase in the total amount of antigen present in the cells. It is of interest, however, that the 250-kD core glycoprotein is the major component immunoprecipitated from the hybrid cells, whereas the higher molecular weight glycoconjugates, although detectable, are minor components.

Table 1. Discordancy analysis for cell surface expression of mel-CSPG and presence of human chromosomes. The reactivity of rodent-human hybrids with monoclonal antibodies B5, 225.28, 9.2.27, and A0122 was determined. Hybrid clones and their human chromosome content have been described in detail (*8, 9*); clones NSK-1, -1s, -2, -3, -4, -5, -6, -7, -9, and -10, as well as CE12 and CE12/6/1 were tested with all four monoclonal antibodies; clones NBE-C2, -D4, -E1, -G1, -H1, -J2, -K1, -M1, and -N1, all grown on ECM-coated substrates, were tested with B5 and A0122; clones AM2, CE25/1, A9/1620CLB, A9/1492/1, and LC1/3/45 were tested with A0122.

Chromosome	Number of discordant hybrid clones			
	B5	225.28	9.2.27	A0122
1	5	2	2	5
2	14	7	7	18
3	12	8	8	14
4	8	4	4	12
5	11	8	8	15
6	10	7	7	12
7	13	10	10	18
8	13	9	9	18
9	15	9	9	20
10	7	4	4	11
11	4	2	2	9
12	7	6	6	11
13	15	9	9	20
14	5	4	4	8
15	0	0	0	0
16	8	4	4	12
17	11	8	8	15
18	16	9	9	20
19	10	8	8	14
20	5	2	2	9
21	5	2	2	9
22	16	8	8	20
X	4	2	2	6
Y	16	9	9	20

To show that mel-CSPG is specifically induced by ECM, we examined NBE hybrids cultured on ECM for changes in the expression of two other cell surface antigens encoded by human chromosome 15—namely, SV13 (105-kD glycoprotein) and F23 (140-kD glycoprotein) (*8*). We found that the eight NBE clones containing human chromosome 15 did not show any changes in F23 and SV13 expression when transferred from plastic dishes to ECM-coated dishes. No changes were observed in the expression of six additional human cell surface antigens encoded by other chromosomes. Extracellular matrix-induced expression of mel-CSPG is also specific with regard to the target cells. A number of mel-CSPG$^-$ human cell lines, including cells of epithelial and hematopoietic origin (*12*), were cultured on ECM and examined for changes in cell morphology and antigen expression. After 24 to 74 hours, some of the cell lines showed slight changes in cell morphology, but in no case did we find induction of mel-CSPG surface expression.

Extracellular matrix is a complex mixture of proteins and its specific effects on cell morphology and expression of differentiated cellular traits in a number of cultured cell types have been established [for review see (*13*)]. However, little is known about surface antigenic systems modulated by ECM, and mel-CSPG is the first antigen shown to be regulated in this way. Induction of mel-CSPG by ECM occurs coordinately with specific changes in cell morphology and substrate adhesiveness, indicating that the ECM signal activates a program of alternative differentiation rather than expression of a single gene product (*14*). It will be important to define the nature of the ECM signal and the ECM receptor and to identify other cell surface antigens regulated by ECM. Expression of mel-CSPG in hybrids derived from mel-CSPG$^-$ human cells (induced by signals provided by the rodent fusion partner) shows that the mel-CSPG gene is not irreversibly inactivated in antigen-negative human cells. Instead, antigen expression is dependent on the specific differentiation program of the human or hybrid cells harboring the mel-CSPG gene. The synthesis of human albumin and other liver-specific proteins in hybrids derived from fusions between human leukocytes or amniocytes and mouse hepatoma cells provides a precedent for the induction of silent traits in rodent-human somatic cell hybrids (*15*).

Although mel-CSPG was initially described on human melanoma cells, other neuroectoderm-derived tumors, including astrocytomas and neuroblastomas, also express this antigen (*3, 4, 6*). In contrast, their nontransformed cellular counterparts—melanocytes, glial cells, and neurons—show no detectable mel-CSPG expression in vivo (*4*). Mel-CSPG$^+$ tumor cells may no longer require extrinsic signals for mel-CSPG expression or, alternatively, tumor cells (or mesenchymal cells present in the tumors) may produce specific matrix components (*16*) that can act as appropriate signals for antigen expression.

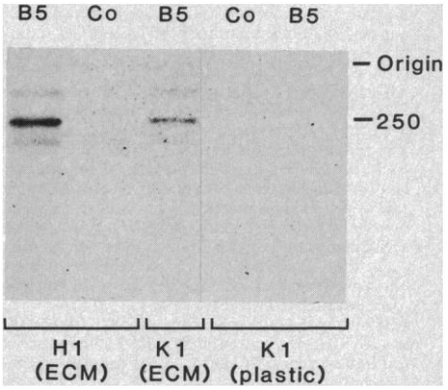

Fig. 3. Fluorograph of immunoprecipitates obtained with monoclonal antibody B5 from extracts of NBE-H1 and NBE-K1 cells and separated on 9 percent sodium dodecyl sulfate–polyacrylamide gels. Cells grown in plastic tissue culture flasks were transferred to new plastic flasks or plastic flasks coated with ECM and cultured in the presence of [^{3}H]glucosamine (New England Nuclear) at 20 μCi/ml for 3 days. Nonidet P40-extracts were prepared as described (*10*), [^{3}H]glucosamine incorporation was determined after precipitation of cellular proteins from cell lysates with 10 percent trichloroacetic acid. Aliquots with equal ^{3}H counts per minute were used in immunoprecipitation tests with antibody B5 (B5) or unrelated control antibody (Co). Molecular weights of immunoprecipitated components were determined as described and are indicated on the right.

REFERENCES AND NOTES

1. R. A. Reisfeld and S. Ferrone, Eds., *Melanoma Antigens and Antibodies* (Plenum, New York, 1982).
2. A. C. Morgan, D. R. Galloway, R. A. Reisfeld, *Hybridoma* **1**, 27 (1981).
3. B. S. Wilson, K. Imai, P. G. Natali, S. Ferrone, *Int. J. Cancer* **28**, 293 (1981).
4. F. X. Real *et al.*, *Cancer Res.* **45**, 4401 (1985).
5. P. G. Natali, A. Bigotti, R. Cavaliere, M. R. Nicotra, S. Ferrone, *J. Natl. Cancer Inst.* **73**, 13 (1984).
6. K. E. Hellstrom *et al.*, *J. Immunol.* **130**, 1467 (1983); A. Giacomino *et al.*, *Cancer Res.* **44**, 1281 (1982).
7. T. F. Bumol, L. E. Walker, R. A. Reisfeld, *J. Biol. Chem.* **259**, 12733 (1984).
8. W. J. Rettig *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6437 (1984).
9. W. J. Rettig *et al.*, *Somat. Cell Mol. Genet.* **10**, 297 (1984); N. C. Dracopoli *et al.*, *ibid.*, p. 475; W. J. Rettig *et al.*, *J. Exp. Med.* **162**, 1603 (1985).
10. J. G. Cairncross *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5641 (1982).
11. Epitope analysis was carried out by testing antibodies 9.2.27, 225.28, B5, A050, A092, A0122, or

unrelated control antibodies for inhibition of binding of [^{35}S]-labeled antibody B5 in a direct radiobinding assay on SK-MEL-13 and LA1-5s target cells. B5 binding was inhibited by 9.2.27, A050, A092, and A0122, but not by 225.28. Antibodies 9.2.27 and A0122 differed markedly in their blocking profiles when tested with different target cells. The epitopes detected by A050, A092, and A0122 could not be distinguished, but the B5 epitope can be distinguished from both 9.2.27 and A0122 epitopes since YH21 Chinese hamster cells are strongly positive for B5, but not for any of the other antibodies to mel-CSPG. Thus, the epitopes detected by B5, 9.2.27, and A0122 appear to be distinct but spatially related, whereas the 225.28 epitope is spatially unrelated.

12. Cell lines tested were colon cancers SW1116, HT29, and SW403; ovarian cancer SK-OV-3; breast cancer Cama; lung cancer SK-LC-9; and leukemias NALL-1 and NALM-16.
13. D. J. Gospodarowicz, *Prog. Clin. Biol. Res.* **145**, 103 (1984).
14. Mel-CSPG is part of a coordinately expressed differentiation program in cultured human neuroblastoma cells. We previously isolated variants from several neuroblastoma lines that are distinguished by an epithelial or fibroblast-like morphology and increased substrate adhesiveness [R. A. Ross, B. A. Spengler, J. L. Biedler, *J. Natl. Cancer Inst.* **71**, 741 (1983)]. These variants are mel-CSPG$^+$, whereas the parental cells with typical neuronal morphology and loosely substrate-adherent growth pattern are mel-CSPG$^-$ (W. J. Rettig *et al.*, in preparation). The growth characteristics of the mel-CSPG$^+$ and mel-CSPG$^-$ human neuroblastoma variants correspond to those of the mel-CSPG$^+$ (ECM) and mel-CSPG$^-$ (plastic) NBE cells, respectively; however, the human neuroblastoma cells do not show morphological interconversion when grown on ECM for up to 7 days (unpublished results).
15. G. J. Darlington, H. P. Bernhard, F. H. Ruddle, *Science* **185**, 859 (1974); J. K. Rankin and G. J. Darlington. *Somat. Cell Genet.* **5**, 1 (1979).
16. A. Oldberg, E. G. Hayman, E. Ruoslahti, *J. Biol. Chem.* **256**, 10847 (1981); C. P. Dietrich, L. O. Sampaio, O. M. S. Toledo, C. M. F. Cassaro, *Biochem. Biophys. Res. Commun.* **75**, 329 (1977); R. V. Iozzo, J. A. Goldes, W. Chen, T. N. Wright, *Cancer* **48**, 89 (1981).
17. We thank R. A. Reisfeld for providing monoclonal antibody 9.2.27, S. Ferrone for providing monoclonal antibody 225.28, and N. C. Dracopoli for the A9/DXB-6 hybrid. Supported in part by National Cancer Institute grant CA-08748, by the Oliver S. and Jennie R. Donaldson Charitable Trust, and by a fellowship from the Deutsche Forschungsgemeinschaft (to W.J.R.).

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Biosynthesis of the *Torpedo californica* Acetylcholine Receptor α Subunit in Yeast

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Yeast cells were transformed with a plasmid containing complementary DNA encoding the α subunit of the *Torpedo californica* acetylcholine receptor. These cells synthesized a protein that had the expected molecular weight, antigenic specificity, and ligand-binding properties of the α subunit. The subunit was inserted into the yeast plasma membrane, demonstrating that yeast has the apparatus to express a membrane-bound receptor protein and to insert such a foreign protein into its plasma membrane. The α subunit constituted approximately 1 percent of the total yeast membrane proteins, and its density was about the same in the plasma membrane of yeast and in the receptor-rich electric organ of *Electrophorus electricus*. In view of the available technology for obtaining large quantities of yeast proteins, it may now be possible to obtain amplified amounts of interesting membrane-bound proteins for physical and biochemical studies.

THE NICOTINIC ACETYLCHOLINE RE-ceptor in the *Torpedo* and *Electrophorus electricus* electroplax is among the best known membrane proteins involved in the transmission of signals between cells (*1–3*). The *T. californica* receptor contains five subunits, two of α, and β, γ, and δ (*4, 5*). The primary structure of these subunits (*6–9*) has been determined by cloning and sequencing of complementary DNA (cDNA) clones. The messenger RNA (mRNA) corresponding to each of the subunits is transcribed in vitro, and the receptor (or individual subunits) is expressed after microinjection (*10*) into *Xenopus* oocytes (*11, 12*). This approach has been used to demonstrate that all four subunits are essential for receptor function (*13*). It has also been used in conjunction with site-specific mutations (*14*), in attempts to locate the functional regions of the α subunit (*10*). We report here that yeast cells transformed with a plasmid containing cDNA for the *T. californica* α subunit synthesize a protein that has the expected molecular weight, antigenic specificity, and ligand-binding properties of the α subunit. Furthermore, this protein is inserted into the yeast plasma membrane.

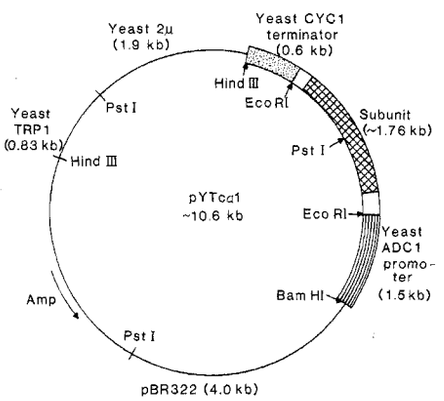

Fig. 1. A yeast plasmid, pYTcα1, that directs synthesis of the α subunit of the *T. californica* acetylcholine receptor in yeast. The 1760-bp cDNA containing the α subunit gene was isolated as an Eco RI fragment from a λ gt-10 library (*24*). This fragment was inserted by ligation into the Eco RI site of pMAC561 to generate pYTcα1. Correct orientation of the α subunit gene with respect to the *ADC1* promoter and the *CYC1* transcription terminator was confirmed by restriction mapping. As indicated, pYTcα1 also contains an origin of replication from the yeast 2-μ plasmid, the wild-type *TRP1* gene, and pBR322 sequences, all derived from pMAC561.

The entire structural gene for the α subunit was previously isolated as a cloned cDNA fragment of 1760 base pairs (bp). This fragment was inserted into the yeast expression vector pMAC561 (*15*) in the correct orientation, downstream of the strong constitutive promoter of the *ADC1* gene and upstream of a transcription termination site derived from the *CYC1* gene. The resulting plasmid, pYTcα1 (Fig. 1), would be expected to direct the synthesis of large amounts of functional mRNA encoding the α subunit in yeast. This plasmid was used to transform the *Saccharomyces cerevisiae* strain TD4 to the Trp$^+$ phenotype.

The expression of cDNA for the acetylcholine receptor α subunit in yeast was first detected by immunoblotting of extracts from transformed cells (Fig. 2). Total cellular proteins were separated by sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoresis and then blotted onto nitrocellulose and reacted with a rat monoclonal antibody (*16*) against the α subunit of the *T. californica* receptor. Immune complexes were detected by successive incubations with rabbit anti-mouse immunoglobulin G (IgG) and ^{125}I-labeled protein A (Fig. 2). Yeast cells that contained the plasmid pYTcα1 (Fig. 2, lanes 11 to 17) contained a protein that was immunoreactive and had the same mobility as the α subunit from *T. californica* membranes (Fig. 2, lane 5). Yeast cells containing pMAC561 without the α

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