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.
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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however, the human neuroblastoma cells do not show morphological interconversion when grown on ECM for up to 7 days (unpublished results).
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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.

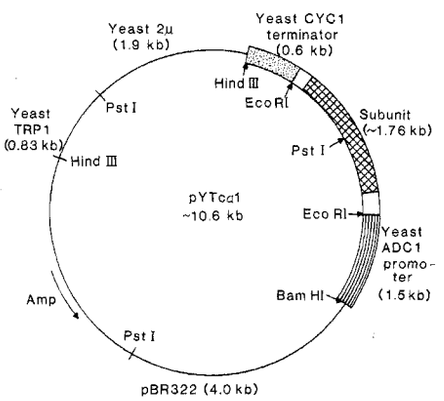

Fig. 1. A yeast plasmid, pYTcα1, that directs synthesis of the α subunit of the *T. californica* acetylcholine receptor in yeast. The 1760-bp cDNA containing the α subunit gene was isolated as an Eco RI fragment from a λ gt-10 library (*24*). This fragment was inserted by ligation into the Eco RI site 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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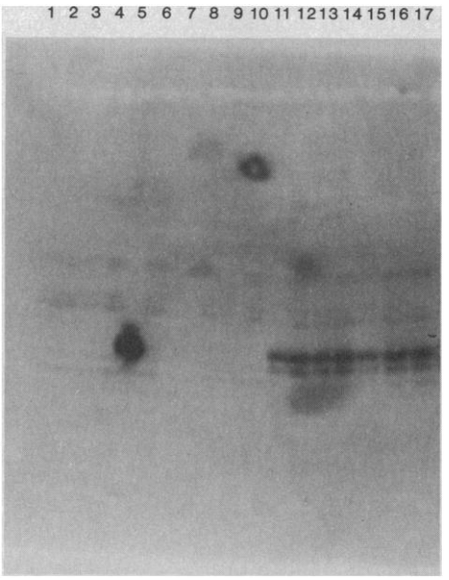

Fig. 2. Detection of the α subunit of the acetylcholine receptor in yeast cells. *Saccharomyces cerevisiae* strain TD4 (*MATA, his-4-519, leu2-3, leu2-112, ura3-52, trpl-289*) was transformed (*25*) with DNA of either pYTcα1 (Fig. 1) or pMAC561 (*15*). Colonies of yeast cells were grown on minimal media containing 2 percent D-glucose, 0.67 percent yeast nitrogen base without amino acids, L-leucine HCl (30 mg/liter), L-histidine HCl (20 mg/liter), and uracil (20 mg/liter). Yeast from 10 ml of culture medium was harvested by centrifugation and lysed by treatment with alkali and mercaptoethanol, and the protein was precipitated by trichloroacetic acid (*17, 26, 27*). Protein from the lysed cells was solubilized in dissociation buffer and separated on a 10 percent SDS–polyacrylamide gel. Proteins (10 to 30 μg) were applied on each lane except for lane 5, which contained about 8 μg of protein from the *T. californica* electroplax membrane containing the acetylcholine receptor (*28*). Lanes 1 to 4, protein from independent transformants containing the vector pMAC561 without the inserted cDNA for the α subunit of the acetylcholine receptor; lanes 6 to 10, protein from yeast cells (strain TD4) containing no plasmid; lanes 11 to 17, protein from independent transformants carrying the plasmid pYTcα1, containing a cDNA insert for the acetylcholine receptor α subunit. The proteins were transferred from the gel to nitrocellulose paper and treated with 5 μl of monoclonal antibody (mab 142) against the α subunit of the acetylcholine receptor. The filters were then reacted with rabbit antibodies to mouse IgG and ^{125}I-labeled protein A (*17*).

subunit (Fig. 2, lanes 1 to 4) and untransformed cells (Fig. 2, lanes 6 to 10) did not contain this protein. Thus, yeast cells can synthesize the α subunit of the *T. californica* acetylcholine receptor.

The cellular location of the α subunit in yeast was studied by fractionation of disrupted cells. The α subunit appeared in the fractions, obtained by differential centrifugation, that contained plasma membrane markers (Fig. 3, B, C, and E). Smaller amounts of the subunit were also present in fractions that were rich in mitochondria and in the pelleted microsomal fraction (Fig. 3, B and D). The supernatant (Fig. 3B, lane 7), devoid of membrane fragments, was free of α subunit.

Insertion of the α subunit into the yeast plasma membrane was confirmed by immunofluorescent detection on intact spheroplasts (Fig. 4). A strong positive reaction was detected after spheroplasts of transformed cells containing pYTcα1 were incubated with an α subunit–specific monoclonal antibody 142 (*16*), washed, and reacted with a second antibody conjugated to fluorescein isothiocyanate (*17*). Yeast cells containing pMAC561 without the α subunit and untransformed cells were not stained by this procedure. Similarly, in control experiments from which the antibody to the α subunit was omitted, no fluorescence was observed. To control for the possibility that antibodies might be able to penetrate the plasma membrane of our spheroplasts, even though they were not permeabilized, spheroplasts were also treated with antibody against porin, a major protein of the outer mitochondrial membrane. In contrast to the result with the antibody to the α subunit, treatment with antibody to porin did not result in positive immunofluorescence, unless the spheroplasts were first permeabilized by treatment with Triton X-100 (Fig. 4). We conclude that at least some of the α subunits

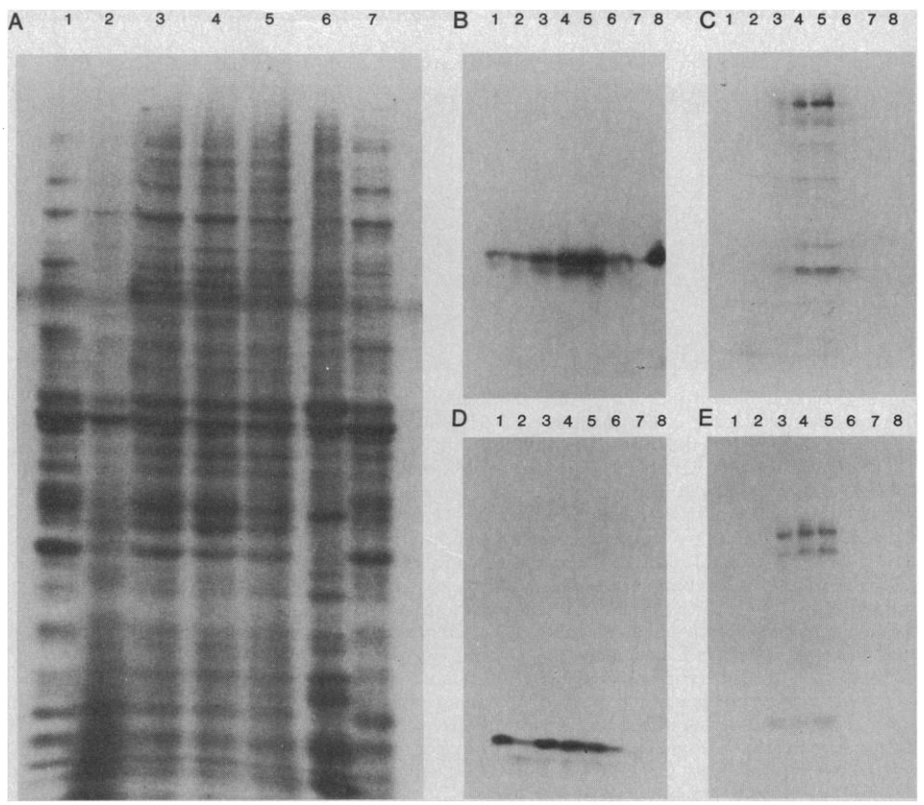

Fig. 3. Distribution of the α subunit of the acetylcholine receptor in various fractions of yeast cells. Yeast cells containing pYTcα1 were grown in 4 liters of medium as in Fig. 2. Subcellular fractions were isolated from spheroplasts that had been prepared by zymolase treatment and were then broken by vortexing in the presence of glass beads. The homogenate was sedimented at 500*g* and the pellet discarded. The supernatant was fractionated at centrifugation speeds and times indicated below. The pellets were solubilized in dissociation buffer, and about 30 μg of protein was applied to each lane of an SDS–polyacrylamide gel. (A) A Coomassie brilliant blue stain of the various fractions: Lane 1, total spheroplast protein; lane 2, 100*g* pellet (10 minutes); lane 3, 3,000*g* pellet (10 minutes); lane 4, 10,000*g* pellet (10 minutes); lane 5, 30,000*g* pellet (20 minutes); lane 6, 200,000*g* pellet (1 hour); lane 7, 200,000*g* supernatant; lane 8, not shown in (A), had 2 μg of membrane protein from *T. californica* membranes containing <0.2 μg of receptor protein (*28*). Similar gels were transferred to nitrocellulose and reacted with different antibodies as in Fig. 2. (B) Monoclonal antibody 142 against the α subunit of the acetylcholine receptor. (C) Antibody against adenosine triphosphatase of the yeast plasma membrane. (D) Antibody against the mitochondrial outer membrane protein, porin. (E) Antibody against three unidentified polypeptides of the yeast plasma membrane. The antibodies used in (C), (D), and (E) were obtained from rabbits and immune complexes were reacted directly with ^{125}I-labeled protein A.

of the acetylcholine receptor that were expressed in yeast were present in the plasma cell membrane. Monoclonal antibody 142 has been shown to bind to the cytoplasmic surface of intact receptors (*18*). Our observation that this antibody can bind to the extracellular surface of α subunits expressed in yeast cells shows that the polypeptide chain of the α subunit does not achieve its native transmembrane orientation.

There is presumptive evidence that post-translational modification of the α subunit has occurred in yeast. The α subunit of the *T. californica* acetylcholine receptor contains a single potential site of *N*-glycosidic linkage, at asparagine residue 141 (*6, 10*). When this residue is converted to aspartic acid, SDS–polyacrylamide gel electrophoresis revealed that the modified α subunit migrated faster than that of the wild type (*10*). The acetylcholine receptor containing the mutated α subunit exhibited barely detectable binding to α-bungarotoxin (*19*). The experiments shown in Figs. 2 and 3 indicate that the mobility of the α subunit made by yeast and of the wild type is identical. The subunit also bound α-bungarotoxin. A K_D value of 0.1 μ*M* was determined (*20*). Toxin binding was completely inhibited by 0.1 m*M* *d*-tubocurarine, which competes with acetylcholine binding to the receptor (*21*). Yeast plasma membrane preparations corresponding to the 3,000*g*, 10,000*g*, and 30,000*g* fractions (Fig. 3) were used in the toxin-binding experiments (*19*). These results are in agreement with previous measurements made with the α subunit isolated from the *Torpedo* spp. acetylcholine receptor (*22, 23*). Each 100 μg of protein of the yeast membrane preparation contained 17 pmol of α-bungarotoxin–binding sites (*20*). With these data and the molecular weight of the α subunit of 50,000 (*6*), it can be calculated that the α subunit of the acetylcholine receptor constitutes approximately 1 percent of the transformed yeast membrane proteins. This density of the α subunit in the yeast plasma membrane is essentially the same as in the plasma membrane of receptor-rich electroplax cells in *E. electricus* (*2*).

These experiments indicate that yeast can synthesize the α subunit of the *T. californica* acetylcholine receptor and that the subunit is then inserted into the plasma membrane. The ability to express the acetylcholine receptor protein in yeast may make it possible to determine structural features of the α subunit important for its insertion into cell membranes and to conveniently do genetic analysis on this protein if its expression has phenotypic consequences on yeast cells.

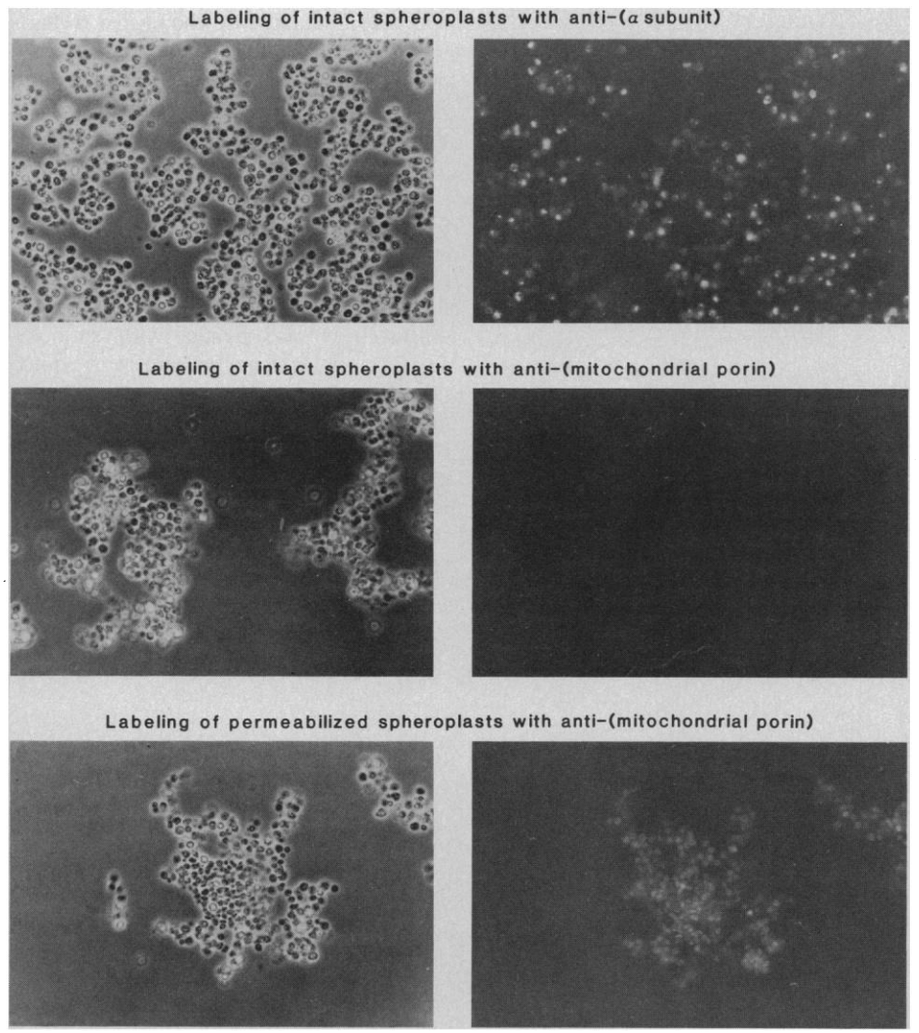

Fig. 4. Detection of the cloned α subunit of the acetylcholine receptor in the yeast plasma membrane by immunofluorescence. The cells containing pYTcα1 (200 ml) were harvested and treated with zymolase to prepare spheroplasts, which were then treated with 3 percent formaldehyde for 30 minutes on ice. The fixed spheroplasts were washed twice with 12 ml of a solution containing 140 m*M* sodium chloride, 25 m*M* tris hydrochloride, *p*H 7.6, and 0.5 percent bovine serum albumin (BSA solution), which was used for all subsequent incubations. The spheroplast preparation was divided into six aliquots. Aliquots 3 and 6 were treated with 1.0 percent Triton X-100 for 10 minutes. The spheroplasts were then suspended in 2 ml of the BSA solution, and aliquots 2 and 3 were incubated overnight with 5 μl of anti-porin that had been raised in rabbit (*29*). Aliquots 5 and 6 were incubated with monoclonal antibody 142 against the α subunit. The fractions were then washed three times with 12 ml of BSA solution; aliquots 1 through 3 were incubated with 20 μl of fluorescein-isothiocyanate–conjugated goat antibodies to rabbit IgG, and aliquots 4 through 6 were incubated with 20 μl of fluorescein isothiocyanate–conjugated rabbit antibodies to mouse IgG. After incubation for 2 hours at room temperature, the spheroplasts were washed three times with 12 ml of BSA solution and resuspended in 0.5 ml BSA solution. Aliquots 1 and 4 gave no detectable fluorescence and are not shown. Figure segments on the left show phase contrast and on the right show fluorescence under the same conditions. Aliquot 6 was treated with Triton X-100 and reacted with antibody against the acetylcholine receptor; the result was similar to that observed with aliquot 5.

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Monooxygenase Induction and Chlorobiphenyls in the Deep-Sea Fish *Coryphaenoides armatus*

JOHN J. STEGEMAN, PAMELA J. KLOEPPER-SAMS, JOHN W. FARRINGTON

Inhibition of liver microsomal ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities by α-naphthoflavone and by polyclonal antibodies to hydrocarbon-inducible cytochrome P-450E from teleost liver indicated a xenobiotic-induced origin of these activities in the deep-sea fish *Coryphaenoides armatus*. Specific recognition of a protein by antibodies to P-450E in an immunoblot assay further indicated xenobiotic-induced cytochrome P-450 in these animals. Levels of apparently induced cytochrome P-450 and monooxygenase activity correlated positively with the tissue content of chlorobiphenyls of known inducing activity, implicating such compounds in biochemical effects occurring in the deep ocean.

REGIONS OF THE DEEP SEA BELOW 1000 m are generally more stable environments than shallower continental shelf and coastal regions (*1*). Recent evidence, however, shows the potential for coupling between surface ocean biological activity and deep ocean organisms (*2*). Previous studies revealed a penetration of the deep sea by foreign compounds originating from land-based activities (*3*). Deep-sea waste disposal now under consideration (*4*) would contribute further to the occurrence of such compounds.

Cytochrome P-450 monooxygenases catalyze transformation of many foreign compounds, leading to detoxification or bioactivation. Cytochrome P-450 isozymes having specific catalytic functions are induced in fish by 3-methylcholanthrene (MC), β-naphthoflavone (BNF), other hydrocarbons, and certain halobiphenyls (*5*). These induced proteins and their activities detected in feral fish in coastal waters have been attributed to the action of such chemicals (*6*). Our analyses of cytochrome P-450 systems indicate that some deep-sea fish have an induced capacity to biotransform xenobiotics and, therefore, that biochemical effects of xenobiotics are now occurring in the deep ocean.

A widely distributed deep-sea fish, the rattail *Coryphaenoides armatus*, was sampled from two sites in the western North Atlantic: near Hudson Canyon on the continental slope off New York and near Carson Canyon off Newfoundland (*7*). The animals were alive on retrieval, were of a similar size (40 to 80 g) at each site, and their gonads were not obviously developed. Genetic distinctions among fish from these sites are not likely to be large (*8*).

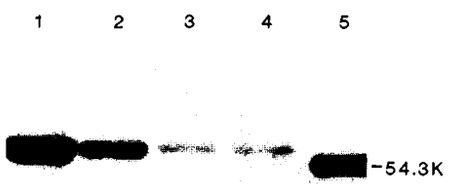

Fig. 1. Immunoblot of *C. armatus* liver microsomes with polyclonal antibody to P-450E. Microsomal proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose (*23*). Nitrocellulose was exposed to antibody to P-450E (1:500) followed by goat antibody to rabbit IgG and rabbit peroxidase-antiperoxidase (Bio-Rad peroxidase staining method). The same amount of microsomal P-450 (20 pmol) was added to lanes 1 to 4. Lane 1, Hudson Canyon fish (EROD, >2.0 pmol/min-pmol P-450); lane 2, Hudson Canyon fish (EROD, 0.8); lanes 3 and 4, Carson Canyon fish (EROD, <0.2 in each). Lane 5 contained 6.5 pmol of purified scup P-450E. Quantitation of cross-reacting material in *C. armatus* was not possible due to lack of standards, but intensity of staining with purified P-450E is linear to about 6 pmol.

Previous studies (*9*) showed that hepatic microsomal preparations of *C. armatus* had active electron transport components and native cytochrome P-450. Hepatic microsomes prepared and analyzed by established methods (*9*) had similar levels of microsomal cytochrome P-450 (about 0.30 nmol/mg) and of reduced nicotinamide adenine dinucleotide phosphate–cytochrome *c* (P-450) reductase activity (90 nmol/min-mg) in fish from both sites.

Ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities, which are catalyzed principally by MC- or BNF-inducible cytochrome P-450 isozymes in fish (*10*), were detectable in all samples but were significantly higher in animals near Hudson Canyon (Table 1). Levels of activity per nanomole of cytochrome P-450 (a measure of catalytic efficiency) in fish taken near Hudson Canyon were comparable in some individuals to the levels in MC-induced fish species from shallow waters, that is, greater than 2 nmol per minute per nanomole of cytochrome P-450 for EROD activity. AHH activity of Hudson Canyon samples was inhibited more strongly by α-naphthoflavone (ANF), a compound that preferentially inhibits catalytic activity of MC-inducible cytochrome P-450 in fish (*6, 10*) and mammals (*11*). By contrast, ethoxycoumarin *O*-deethylase (ECOD) activity differed to a lesser extent between these groups. In other fish, ECOD activity is catalyzed as much as 50 percent by other, noninducible isozymes (*12*).

The conclusion that the EROD and AHH activities in *C. armatus* reflect the presence of induced cytochrome P-450 was strongly supported by analysis with polyclonal antibodies to the MC-inducible liver cytochrome P-450 (cytochrome P-450E) (*12*) from the teleost *Stenotomus chrysops*. These antibodies inhibit EROD activity, and cross-reactivity is evident in enzyme inhibition and immunoblotting with similarly induced isozymes in other fish species (*12, 13*). Antibodies to P-450E preincubated with *C. armatus* liver microsomes inhibited EROD activity progressively in titration, up to 45 ± 3 percent (n = 3) at a ratio of 60 μg of purified immune immunoglobulin G (IgG) per picomole of P-450, compared to activity with preimmune IgG (*14*). Immunoblotting of *C. armatus* microsomes revealed that antibodies to P-450E recognized a protein with a molecular weight of about 55,000 (55K), in the range (54K to 58K) of

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