(*20*) and 100 to 300 kN/m^2 as the maximum force-generating capability of the individual cells.

The TnC in cardiac muscle might respond differently from that in fast twitch skeletal muscle in several respects. One of the two trigger sites (the low-affinity Ca^{2+}-specific site I) in CTnC has 7 of the 12 amino acid residues replaced (*13*) in the Ca^{2+}-binding loop (*21*), modifying the Ca^{2+} coordination in the site under normal conditions, and this may be important in producing high length-induced Ca^{2+} sensitivity in cardiac muscle. There are also other minor replacements in the CTnC molecule, but their functional significance is unknown. How the length signal is transmitted to the TnC moiety in the cardiac muscle is also unknown. One possibility is that, as in skeletal muscle, a third set of filaments (containing titin, also called connectin) in the cytoskeletal matrix connects the myosin filaments to the Z-lines at the end of the sarcomere (*22*). The stress in the titin filament induced by sarcomere length would have to be communicated to the regulatory proteins if titin did play a specific role in the Starling mechanism. Alternatively, steric rearrangements within the myofilament lattice below 2.4 μm might selectively reorder the thin filaments in the heart muscle through the altered electrostatic or mechanical factors (*23*) and thereby also affect the regulatory proteins. Further investigations will be necessary to clarify these issues.

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Cone Cell–Specific Genes Expressed in Retinoblastoma

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Retinoblastoma, an intraocular tumor that occurs in children, has long been regarded, on the basis of morphological criteria, as a malignancy of the photoreceptor cell lineage. Here it is shown that when this tumor is grown in vitro, the cells express highly specialized photoreceptor cell genes. Transcripts for the transducin alpha subunit, $T_{C\alpha}$, which is specific to the cone cell, as well as transcripts for the red or green cone cell photopigment, were found in seven out of seven low-passage retinoblastoma cell lines. No marker genes specific to rod cells were expressed, suggesting that retinoblastoma has a cone cell lineage.

RETINOBLASTOMA (RB) IS AN INTRAOCULAR tumor that occurs in children. The neoplasm develops early in life on a sporadic or hereditary basis and may be unilateral or bilateral, with the hereditary form being mainly bilateral (*1*). A chromosomal abnormality has been described for both forms of RB (*2*), and a gene in the RB locus was recently cloned (*3*).

The tumor is derived from neuroectodermal cells, but its cell of origin has not been unequivocally identified. Primary tumors are composed of anaplastic cells with little cytoplasm, and morphologically differentiated structures (fleurettes and Flexner-Wintersteiner rosettes) are found that express structural characteristics of mature photoreceptor cells (*4*). A bidirectional differentiation potential to photoreceptor cells or glial cells, or both, has been postulated on the basis of studies with the Y79 cell line (*5*).

Rod and cone cells are the photoreceptors of the mammalian retina. They express the photopigment genes (rhodopsin and color photopigments) (*6*) that have been isolated from the human genome (*7, 8*). Photopigment photolysis is coupled to cyclic guanosine 5′-phosphate metabolism through a membrane-associated retinal G protein, transducin (*9–11*), and results in a hyperpolarization of the photoreceptor cell membrane (*12, 13*). Bovine complementary DNAs (cDNAs) specific for the rod cell ($T_{R\alpha}$) and cone cell ($T_{C\alpha}$) alpha subunits of transducin have been isolated (*10, 14, 15*).

Here we show that cultured RB cell lines established from individual patients express the red or green photopigment gene, or both genes, but not the gene coding for rhodopsin. Concomitantly, the cells express the cone cell $T_{C\alpha}$ subunit but not the rod cell $T_{R\alpha}$ subunit of transducin. We therefore postulate that RB may be a neoplasm of the cone cell lineage.

Previously isolated RB cell lines that show

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various degrees of morphological differentiation in vitro were grown in mass cultures (*16*). These cell lines represent early-passage tumor populations, which represent a total culture period of less than 12 months. We investigated the expression of the various transducin genes by means of Northern blot analysis. Bovine cDNA probes for the $T_{R\alpha}$ and $T_{C\alpha}$ (*15*) were used since human probes were not available. The RNAs from seven RB lines were hybridized with the $T_{C\alpha}$ subunit cDNA, and two transcripts were detected in the RNAs from RB cell lines but not in RNA from an osteosarcoma cell line (HTLA145) (*17*) (Fig. 1). In RB cells, as in bovine retina, the $T_{C\alpha}$ probe detected a high and a low molecular weight transcript; the low molecular weight message was generally expressed at a higher level. The intensity and electrophoretic mobility of the $T_{C\alpha}$ transcripts in different RB cell lines varied, although equal amounts of total RNA were present when the RNAs were hybridized with the β subunit cDNA probe, which is expressed in all tissues. The extent of expression varied also with culture conditions but could not be correlated with the morphology of the cells, suggesting that expression of $T_{C\alpha}$ is independent of Flexner-Wintersteiner rosette differentiation in vitro.

The Northern blots hybridized with the $T_{C\alpha}$ probe were then used to study the expression of the gene for the $T_{R\alpha}$ subunit. In none of the seven low-passage RB cell lines could we detect transcripts for the rod cell–specific $T_{R\alpha}$. Since Southern blot analysis revealed the presence of the $T_{R\alpha}$ gene in all cell lines tested, we conclude that these cultured RB cell lines express only $T_{C\alpha}$.

The β subunit (T_β) was expressed as two distinct messages in all RB cell lines tested, as has been found in all tissues thus far examined (*18*). A transcript (0.7 kb) for the gamma subunit (T_γ) (Fig. 2) was found in four out of seven RB lines (Table 1) when a bovine cDNA probe was used (*19*); however, no expression was found in the HTLA145 cell line. The intensities and electrophoretic mobilities of the transcripts for T_γ also varied in different cell lines. However, the message size was similar to that found in the mature bovine retina (*20*).

We also investigated the presence of transcripts for the different photopigments in RB cell lines since transducin and pigment proteins are components of the same signal transduction system. In Northern blots used for the analysis of $T_{C\alpha}$ gene expression we did not detect a message for the opsin gene with the bovine opsin cDNA probe (*21*), although strong hybridization with rabbit retina RNA occurred. Southern blots revealed the presence of the opsin gene in all RB lines tested. In seven out of seven early-passage RB lines a transcript for the red or green or both color pigment genes but not for the blue pigment gene was found (Fig. 3). The message size was similar to that found in the mature retina (*8*), and expression levels varied between individual cell lines but could not be correlated with the morphology of the cells. A summary of the analysis is given in Table 1.

The presence of transcripts for gene products involved in cone cell signal transduction is a marker for a lineage analysis of RB and may provide a molecular clue to its developmental origin. The expression of such cone cell specific marker genes therefore suggests that RB is committed within the cone cell lineage, but morphological observations indicate that RB does not represent a terminally differentiated cell (*5, 16*). Transcripts of rod cell–specific genes, if present, were below the limit of detection relative to cone cell markers. In vitro selection for cone cell precursors in RB seems unlikely since these tumors represent early cell passages; however, it is known that chicken cone cells do survive in culture whereas rod cells do not (*22*). Absence of any RB with blue pigment expression may be due to the combination of the small sample size and the low number of blue cones in the human retina (*23*).

This study demonstrates the expression of cone-specific genes in cultured RB. These cells may therefore be an attractive system in which to study photoreceptor development.

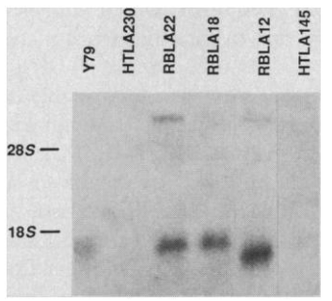

Fig. 1. Expression of $T_{C\alpha}$ subunit in cultured RB cells. RB cell lines of passage 15 through 25 that were in culture less than 12 months were grown as described (*16*). Cells were pipetted off the feeder layers, centrifuged, and lysed in lysis buffer (0.14*M* NaCl, 0.01*M* tris-HCl, *p*H 7.4, 1.5 m*M* $MgCl_2$, and 0.5% NP-40). Nuclei were centrifuged (1500*g*), and the supernatant was extracted three times with phenol and then once with a mixture of chloroform and isoamyl alcohol (24:1 by volume). Total cellular RNA (30 μg per lane) was electrophoresed on a 1% agarose-formaldehyde gel and transferred to Biodyne (ICN), and filters were baked at 80°C for 2 hours. Filters were prehybridized and hybridized in 50% formamide, 5× standard saline citrate (SSC), 1× Denhardt's (0.02% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin), 20 m*M* sodium phosphate, *p*H 6.5, 0.1% SDS, and denatured salmon sperm DNA (250 μg/ml) at 42°C. Filters were washed at a final stringency of 0.3× SSC at 65°C. The $T_{C\alpha}$ cDNA probe consisting of a 1.7–kilobase pair (kbp) Hind III fragment (*15*) was radiolabeled with [^{32}P]deoxycytidine triphosphate (ICN), according to Feinberg and Vogelstein [(*24*), see addendum]. Cell lines: Y79, RB cell line; HTLA230, neuroblastoma; RBLA22, RBLA18, and RBLA12, low-passage RB cell lines; and HTLA145, osteosarcoma.

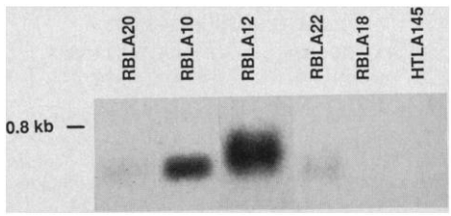

Fig. 2. Expression of retinal transducin T_γ in RB cells. The two 0.2-kbp Eco RI fragments from the bovine retinal transducin T_γ cDNA (*19*) were used and hybridized to RB RNA previously analyzed for the $T_{C\alpha}$ subunit, as described in the legend to Fig. 1.

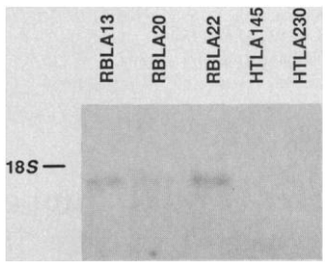

Fig. 3. Expression of red or green color photopigment gene in cultured RB cells. Total cellular RB RNA was hybridized with the human cDNA probe for the red photopigment clone hs7 (*8*), as described in the legend to Fig. 1.

Table 1. Summary of transducin gene and photopigment gene expression in RB cells in vitro. ND, not determined; +, detectable expression; −, no detectable expression.

Cell line	$T_{R\alpha}$	$T_{C\alpha}$	T_γ	T_β	Red or green photopigment	Blue photopigment	Rhodopsin
RBLA10	−	+	+	+	+	−	−
RBLA12	−	+	+	+	+	ND	−
RBLA13	−	+	ND	+	+	ND	−
RBLA18	−	+	−	+	+	−	−
RBLA19	−	+	−	+	+	−	−
RBLA20	−	+	+	+	+	−	−
RBLA22	−	+	+	+	+	ND	−
Y79	ND	+	−	+	ND	ND	−

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Mussel Growth Supported by Methane as Sole Carbon and Energy Source

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Symbioses between chemoautotrophic bacteria and several specialized marine invertebrates are well documented. However, none of these symbioses have been demonstrated to provide sufficient energy and carbon to the host to enable it to grow. Growth rates of seep mussels collected from hydrocarbon seeps off the coast of Louisiana were measured in a controlled environment where methane was the sole carbon and energy source. The growth rates increased to a maximum of 17.2 micrometers per day in response to methane and approached zero in the absence of methane. These mussels contain methanotrophic symbiotic bacteria in their gills, which suggests that these bacteria provide their hosts with a net carbon flux originating from methane.

THE OCCURRENCE OF SYMBIOTIC chemoautotrophic bacteria that reside in highly specialized tissues of certain marine invertebrates was first described as occurring in hydrothermal vent communities and since then in many other diverse reducing marine environments (*1*). These bacteria typically oxidize reduced sulfur compounds from their environment and use the energy obtained to fix carbon dioxide from the surrounding seawater (*2, 3*). These chemicals can be formed either geothermally from seawater sulfate, as at the hydrothermal vents, or biologically through sulfate-reducing bacteria in the sediments, as in mudflats or hypoxic deep-sea basins (*4, 5*). Another source of energy and carbon has recently been demonstrated for mussels associated with hydrocarbon seepage sites on the Louisiana slope in the Gulf of Mexico (*5–7*) at the base of the Florida Escarpment (*8*) and for a small pogonophoran from the Skagerrak (*9*). These animals contain methanotrophic symbionts. The symbionts in the gill cells of the mytilids (*6, 8*) and the trophosome cells of the pogonophorans (*9*) contain stacked internal membranes characteristic of type I methanotrophs. Furthermore, enzymatic tests, stable isotope determinations, net methane uptake studies, and the incorporation of ^{14}C-labeled methane indicate that these symbioses are methanotrophic (*6–8*). The methane necessary for the support of this metabolism in the mussels in situ originates either from hydrocarbon sources at the oil seeps or from biological processes at the Florida Escarpment communities (*4, 5*).

For both chemolithoautotrophic and methanotrophic associations, researchers have proposed (*2, 3, 6*) that at least part of the nutritional requirements of the respective hosts is supplied by the bacteria. Some host animals have entirely lost the ability to take up and digest external food. The vestimentiferan tube worms, the pogonophorans, many oligochaetes of the subfamily Phallodrilinae, and several bivalves of the Solemyidae have lost their digestive systems and must depend on an alternative nutritional source—most likely the symbiotic bacteria (*10*). Similarly, most other bivalves known to contain chemoautotrophic symbiotic bacteria are characterized by a reduced digestive system (*10, 11*). Other indirect evidence for the importance of bacterial carbon to the hosts includes studies in which stable isotope ratios ($^{13}C/^{12}C$) in symbiont-containing animals were measured. Comparisons of paired tissues from individual animals of a variety of symbiont-containing species, including the mussel species used in our study, showed little variation, indicating the importance of symbiont carbon to the host (*5, 6, 12*). This finding is especially convincing in the case of animals containing methanotrophic symbionts because of the negative $^{13}C/^{12}C$ associated with methane. Transfer of symbiont carbon to the host can be accomplished in several ways: through digestion of the bacteria by host lysosomes or through the translocation of part of the fixed carbon from the bacteria to the host. The first strategy has been proposed in the symbioses of the hydrothermal vent tube worm *Riftia pachyptila* by Bosch and Grasse (*13*), who document an intracellular degradation of symbiotic bacteria. Hand (*14*) and Giere and Langheld (*15*) observed similar phenomena in the bacteriocytes of *Riftia pachyptila* and in the oligochaete *Phallodrilus leukodermatus*, respectively. The second proposed strategy of nutrient transfer is the translocation of reduced organic material from the bacteria to the host, as was shown with radiolabeled bicarbonate in *Solemya reidi* (*16*). However, it has not been demonstrated that the hosts can grow when provided only with an inorganic chemical as a bacterial energy source. We report here that mussels harboring methanotrophic bacteria as symbionts (*17*) grow when supplied only with methane in the seawater.

Growth has been considered an excellent

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