surface chemical conditions conducive to specific associations with bacteria. Chemical gradient formation and patchiness near surfaces promote the establishment of physiologically diverse epiphytes, including pseudomonads, whose individual metabolic characteristics lead to enhancement of a localized metabolic process (N_2 fixation) in host cyanobacteria. Parallel associations between vegetative cells and bacterial populations may specifically serve to enhance photosynthetic production by recycling CO_2 and inorganic nutrients (*11*).

Although chemotaxis has been shown to play a role in promoting diverse aquatic algal-bacterial associations, this process may also be instrumental in establishing specific abiotic particle-bacterial interactions in marine and freshwater habitats. Both habitats represent very dilute pools of dissolved energetic and nutritional growth substrates, but such substrates are known to be concentrated on particle surfaces (*12*). The ability to chemotactically sense and seek nutrient-enriched surfaces would offer a distinct advantage under nutrient-limited conditions. Chemotaxis also complements other means of initiating bacterial attachment, including electrostatic forces, random encounters, and mutual accumulation of particles and bacteria along vertical density gradients in the water column. However, in contrast to these mechanisms, chemotaxis offers selectivity based on biologically active molecules released (by diffusion or excretion) from particle surfaces. A great deal of variability in types and magnitudes of microbial colonization has been reported among diverse suspended particles (*12, 13*). Such variability may in part be due to diverse chemotactic agents characterizing the spectrum of biotic and abiotic surfaces present in natural waters.

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A Hydrophobic Transmembrane Segment at the Carboxyl Terminus of Thy-1

Abstract. *The mode of integration of the glycoprotein thy-1 within the membrane has been controversial due to an apparent lack of a transmembr hydrophobic segment. Rat and mouse complementary DNA and genomic clo encoding the thy-1 molecule have been isolated and sequenced. These studies h enabled us to determine the intron-exon organization of the thy-1 gene. Furtherm they have revealed the existence of a sequence which would encode an extra segn (31 amino acids) at the carboxyl terminus of the thy-1 molecule. These extra an acids include a 20–amino acid hydrophobic segment which may be responsible integration of thy-1 within the plasma membrane.*

Thy-1 is a membrane glycoprotein found predominantly on the cell surface of thymocytes and brain cells (*1, 2*). Originally identified in mice, proteins similar to thy-1 are present in many species (*3–6*) although the distribution of thy-1 among hematopoietic cells seems to vary (*7, 8*). Thy-1 proteins isolated from rat and mouse brains have been sequenced and consist of a protein moiety of 111 and 112 amino acids, res tively (*9, 10*). These sequences v lacking a hydrophobic segment, whi necessary for integration of thy-1 wi the lipid bilayer of the membrane. has prompted speculation that thy- covalently linked to some hydroph component such as glycolipid which chors the thy-1 molecule to the m brane. The DNA sequence analyse

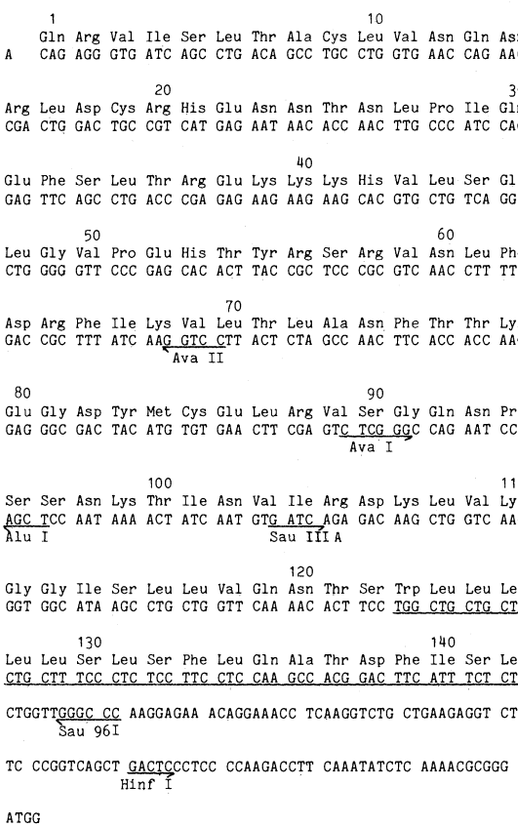

Fig. 1. The nucleotide sequence of the cDNA insert of the thy-1 cDNA clone pT86 and the predicted amino acid sequence of the complete rat thy-1 antigen. The DNA of pT86 was cleaved with restriction endonucleases (BRL) and fragments corresponding to the insert were purified by polyacrylamide gel electrophoresis and electroelution. Purified fragments were treated with calf intestinal alkaline phosphatase (Boehringer), labeled at both 5′ ends with T4 polynucleotide kinase plus [γ-^{32}P]ATP (*11*), and cleaved secondarily to generate subfragments with only one labeled end. The restriction enzyme sites shown and both Pst I sites are those that were labeled at the 5′ end. The subfragments were separated by acrylamide gel electrophoresis, electroeluted, and subjected to partial chemical degradation sequence analysis (*15*). Both strands of the insert cDNA were sequenced. The hydrophobic 20–amino acid segment is underlined and the termination codon is indicated by an asterisk.

ported here have revealed a hydrophobic region (amino acids 124 to 143) which probably represents the transmembrane segment responsible for anchoring thy-1 to the cell.

We previously isolated a rat complementary DNA (cDNA) clone, pT64, which encodes part of the thy-1 molecule (*11*). Analysis of this clone allowed us to define a signal peptide of 19 amino acids as well as part of the 5′ untranslated region of thy-1. Unfortunately, this clone terminated prematurely at amino acid 103 which prevented us from defining the carboxyl end of the molecule. After screening a second rat thymocyte cDNA library using the entire Pst I insert of pT64 as a probe, we obtained an additional thy-1 cDNA clone, pT86. The sequence of the insert from this clone (Fig. 1) started at amino acid 1 and was in agreement with the DNA sequence of pT64. Furthermore, it encoded the entire sequence of 111 amino acids of the thy-1 molecule which was previously obtained by conventional protein sequencing methods. The reading frame encoding this sequence continued on for an additional 31 amino acids before a termination codon, TGA (T, thymine; G, guanine; A, adenine), was encountered. This codon was followed by a 3′ untranslated region of 119 nucleotides before the deoxyguanylate-deoxycytidylate (dG-dC) homopolymeric tail was observed. Within these 31 amino acids there was an extremely hydrophobic stretch of 20 amino acids (including six consecutive leucine residues), which strongly resembled the transmembrane segments found in other membrane proteins. In order to determine whether these extra 31 amino acids were also present in the normal thy-1 gene, rat and mouse genomic libraries were screened with the thy-1 cDNA clone, and positive clones were analyzed in terms of restriction enzyme digestion products and DNA sequence. The coding sequence of the mouse thy-1 gene (Fig. 2) was distributed among three exons; the first one shown (actually the second exon of the gene since an intron is present within the 5′ untranslated part of the gene) encoded part of the 5′ untranslated region and the first 12 amino acids of the signal peptide. This was followed by an intron of 590 nucleotides and then by another exon encoding the remainder of the signal peptide and amino acids 1 to 106 of the mature thy-1 protein. After this exon, there was an additional intron of 386 nucleotides and an additional exon encoding amino acids 107 to 143 plus the termination codon, TGA. A polyadenylation signal, AATAAA, is located 1110 nucleotides downstream from the termination codon. The organization of the rat thy-1 gene is like that of the mouse gene, including the presence of an extra 31 amino acids in the third coding exon (*12*).

Hybridization analyses of rat and mouse DNA and RNA ruled out the possibility of a second thy-1 gene encoding a molecule of 112 amino acids or,

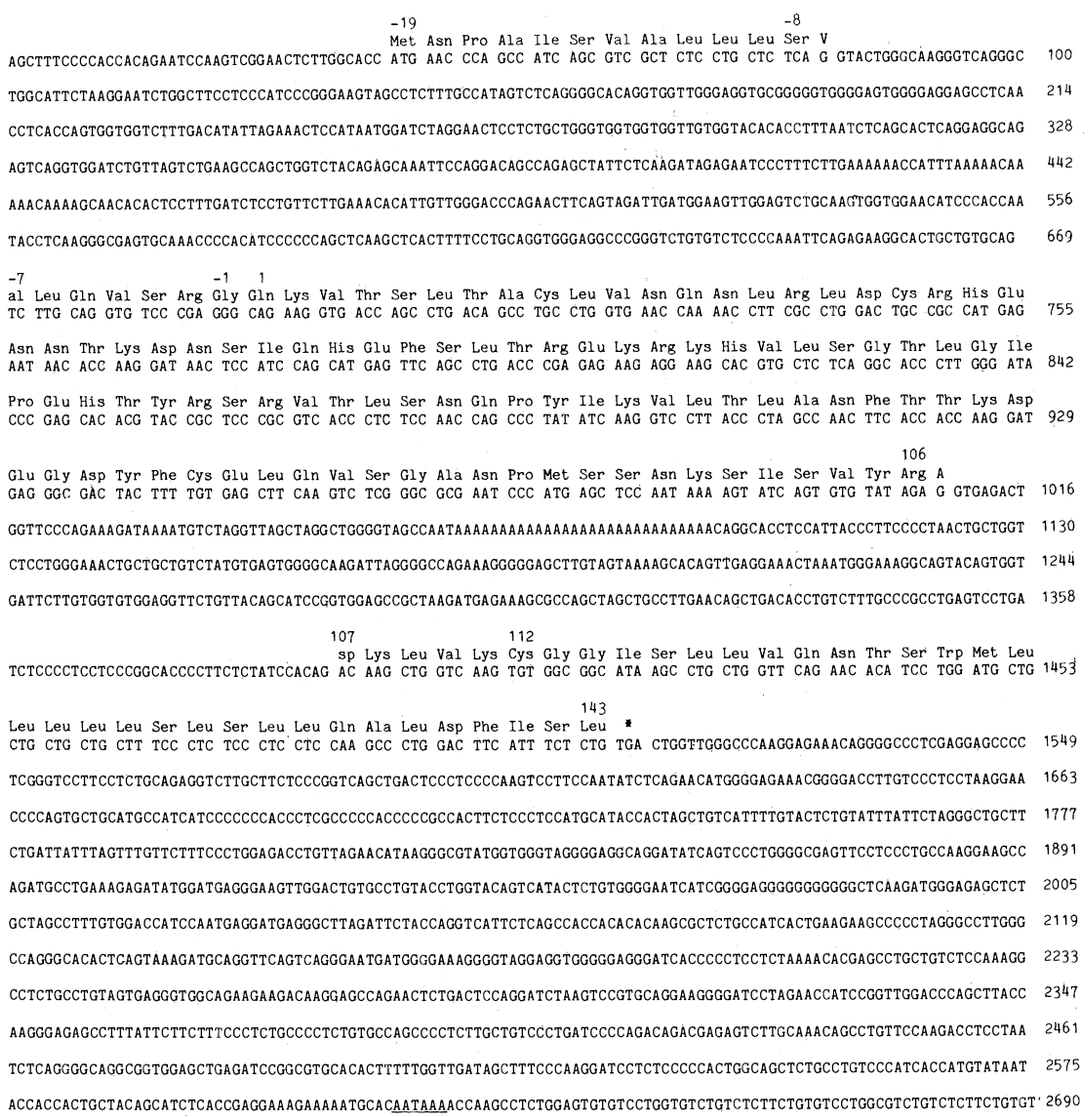

Fig. 2. Nucleotide sequence of the mouse thy-1 gene. A genomic library was prepared in the cosmid vector c2RB (*16*) with mouse (C57BL/6) DNA partially digested with Sau IIIA. The genomic library was screened with a nick-translated thy-1 cDNA probe and two thy-1 genomic clones were obtained. The thy-1 gene was subsequently subcloned into the Eco RI site of pBR322 and sequenced (*15*). The termination codon is indicated by an asterisk.

alternatively, differential processing of a thy-1 nuclear transcript giving rise to a second messenger RNA (mRNA) encoding a thy-1 molecule of 112 amino acids. Only a single thy-1 gene was observed in both the rat and mouse genome and only a single thy-1 mRNA species of approximately 1.85 kilobases (kb) in brain and thymus tissue was detected (*12*). This mRNA hybridized to a nick-translated DNA fragment corresponding to amino acids 107 to 143 of the thy-1 molecule (*12*) and thus included the extra 31 amino acids observed in the cDNA and genomic clones. There is no evidence for a second smaller mRNA encoding a shorter thy-1 molecule.

The discrepancy between the DNA sequence data and the protein sequences of Williams and Gagnon (*10*) may be explained in either of two ways. One possibility is that, although the purified thy-1 molecule is 143 amino acids long, the hydrophobic peptide containing the extra 31 amino acids was lost during the preparation and purification of the peptide fragments that were used for sequencing. Alternatively, the discrepancy between the DNA and protein data may reflect a processing step in which the newly synthesized thy-1 molecules are cleaved to yield mature molecules of a different size. To further explore this possibility, "pulse-chase" experiments were performed as follows. Cells from a murine thymoma, BW5147, which expresses thy-1 on the cell surface were labeled with [^{35}S]methionine for 5 minutes, followed by incubation with unlabeled methionine for various periods of time. Included in these experiments was the compound deoxymannojirimycin which inhibits the cleavage of mannose residues from *N*-linked glycans of thy-1 and consequently simplifies the patterns observed (*13*). Immunoprecipitation of the thy-1 molecule, with a rabbit antiserum to rat thy-1 antibody (*14*) that cross-reacts with murine thy-1, revealed that thy-1 was initially present in two forms of different molecular weights and that within 10 minutes the larger form was converted to a smaller one (Fig. 3). To determine whether this conversion was due to cleavage of the carboxyl terminal 31 amino acids from the thy-1 molecule, pulse-chase experiments were performed with [^{3}H]tryptophan. Since tryptophan is present only in the carboxyl terminal 31 amino acids (at position 124) and absent from the rest of the molecule, cleavage of this extra 31 amino acid stretch would result in the production of an unlabeled thy-1 molecule. Although incorporation of [^{3}H]tryptophan is low, both molecular weight forms were visible when thy-1 was labeled with [^{3}H]tryptophan (Fig. 3); furthermore, the thy-1 molecule was visible even after labeling was followed by an extensive chase (45 minutes in the presence of unlabeled tryptophan) indicating that the mature thy-1 molecule extends beyond the 112 amino acids proposed by Campbell *et al.* (*9*) and at least to amino acid 124. Our inability to detect any other conversion step even after a 90-minute chase (data not shown) suggests that the mature thy-1 molecule has a size consistent with that predicted from the cDNA and genomic data and that its mode of integration in the membrane is via the hydrophobic stretch of 20 amino acids present at the carboxyl terminus.

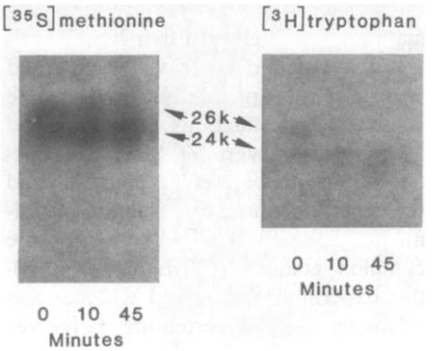

Fig. 3. Biosynthesis of thy-1 in the BW5147 murine thymoma cell line. BW5147 cells were transferred to methionine- or tryptophan-free medium (5×10^6 cells per microliter), incubated for 60 minutes, and exposed to [^{35}S]methionine or [^{3}H]tryptophan at a final concentration of 100 and 250 μCi/ml, respectively. After 5 minutes, nonradioactive amino acid was added to a concentration of 1 m*M* (zero time point). The oligosaccharide cleavage inhibitor deoxymannojirimycin was included during the preincubation period and was continuously present thereafter. Samples (5×10^6 cells) were withdrawn at the time points indicated and processed for immunoprecipitation. Immunoprecipitates were analyzed by sodium dodecyl sulfate–polyacrilamide gel electrophoresis (12.5 percent gel).

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The Goldfish as a Retinex Animal

Abstract. *In an experiment designed to test color constancy in a situation comparable to that used in E. H. Land's experiments with human observers, goldfish were trained to approach a particular color within a richly colored but variable "Mondrian" background. They retained the ability to identify colors accurately even when the spectral composition of the illuminant was radically altered in generalization tests. Since the behavior of fish resembles that of human beings in these tests, Land's retinex theory seems to apply to a relatively primitive vertebrate as well as to humans.*

A key issue in the study of color vision is that colors of objects can be identified despite large changes in illumination conditions, which induce large variations in the spectral composition of light reflected from each object within a scene. This well-known color constancy effect presumably derives from a mechanism that computes relations between spectral features of an object and its surroundings. As part of a long series of observations on such phenomena, Land (*1*) and his colleagues have noted that variations in spectral composition and intensity as