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.

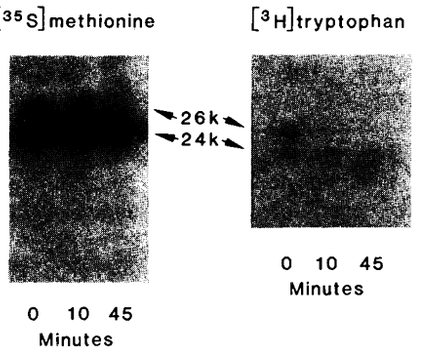

Fig. 3. Biosynthesis of thy-1 in the BW5147 murine thymoma cell line. BW5147 cells were transferred to methionine- or tryptophan-free medium (5×10^6 cells per microliter), incubated for 60 minutes, and exposed to [^{35}S]methionine or [^{3}H]tryptophan at a final concentration of 100 and 250 μCi/ml, 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

far as 30° away from a test area can determine its color as much as nearby objects do (*2*). Such experiments have led to the retinex theory of human color vision (*1*), which has shown wide predictive power (*3*).

The term "retinex" has been left intentionally indeterminate with respect to the locus of computation in the brain. Since retinal receptive field interactions appear too restricted to mediate the wide-field comparisons shown by human subjects, however, most physiologists would be inclined to look for a cortical locus of spatial interactions (*4*). In the anesthetized rhesus monkey, the output of the retinex processing seems to be readable in single neurons of Zeki's visual area IV (*5*). When a multicolored "Mondrian" image is fixed on the retina, these cells give reports on local colored areas in correspondence with human observations. Further evidence that these comparisons are based on a cortical, but not retinal, mechanism derives from a recent demonstration that normal, but not split-brain, persons can use a multicolored Mondrian stimulus in one hemifield as a component of the computation in determining the color of a paper located in the opposite hemifield (*2*).

Another approach to neural modeling of the retinex theory is to find an organism with a less elaborate visual architecture than a primate whose behavior obeys predictions from this theory; for example, are teleost fishes (animals without a recognizable neocortex) retinex animals? Along with birds and primates, several teleost fishes have excellent trichromatic color vision (*6*), and some teleosts apparently see the same color-contrast effects as those reported by human observers (*7*). Since the spectral sensitivities of retinal pigments and the center-surround organization of retinal ganglion cells are well known for the common goldfish (*Carassius auratus*), this experiment addressed whether this relatively simple vertebrate perceives colors as constant under a variety of critical illuminated variations as the human observer does.

I trained fish to approach a colored object for food reward under even illumination with a specified white light and to ignore other colors relatively near in hue. When the task was learned, the fish was tested to see whether it would generalize this training to radically different illumination conditions [such as those used in assessment of human color perception (*1*)] and behave as if it sees an unchanged color. Alternatively, the fish may be too simple an operator to make the computations posited by the retinex model and may instead be guided chiefly by the spectral composition of light reflected from a particular colored area.

To my knowledge, only one study (*8*) has been directed toward the operation of color constancy in fish species. In 1923, Burkamp trained fish of three different species to select feeding cups of a particular color and to ignore other hues, including a large variety of gray shades. He then tested for specific color selection after interposing various gelatin filters in the path of light entering the window. Although this study, exemplary for its time, suggested the operation of color constancy in fishes, three weaknesses prevented knowing how good their constancy mechanism really is. (i) The fish made many errors in choosing gray cups when novel illuminants were used, which would not have been expected in a human observer. (ii) No quantitative spectral information was available regarding the object colors or the gelatin filters modifying the wavelength composition of the scene. (iii) Burkamp adapted his fish gradually to each novel lighting condition. My experiment was designed to measure, in highly trained fish, the precise changes in spectral composition of each area of the Mondrian in terms of light reaching the fishes' eyes and to test color memory after abrupt changes in composition of the illuminant.

Goldfish learned to approach an aperture within a white wall at the end of the training tank, behind which was inserted a colored paper (Fig. 1), to obtain a small piece of beef liver from a pipette. Green-positive fish also learned to ignore blue-green and yellow-green paper, and yellow-positive fish learned to ignore yellow-green and orange apertures. The fish continued to perform this simultaneous discrimination after we introduced a Mondrian background (*9*) composed of a variety of brightly colored papers (Fig. 1 and cover) in place of the white background. Three fish attained a stable performance (at least 90 percent correct over 60 trials) on the three-color task with the Mondrian background.

The test tank was moved into a dark room where the Mondrian could be evenly illuminated by projecting light through a water prism. Training continued under a condition in which the separate intensities of red (690 nm), green (545 nm), and blue (450 nm) filtered lights (*10*) were adjusted to produce equal intensities of each light source reflected from a medium gray patch on the Mondrian. Each colored patch was viewed from the opposite end of the tank through a telephotometer (*10*); and measured long, middle, and short-wavelength light reflected from various areas of the Mondrian as well as from the colored patches to be discriminated behind the apertures. During this training phase (condition 1), the positive green stimulus was presented with either (i) blue-green plus yellow-green, (ii) blue plus blue-green or (iii) yellow plus yellow-green; and the positive yellow stimulus was presented with either (i) green

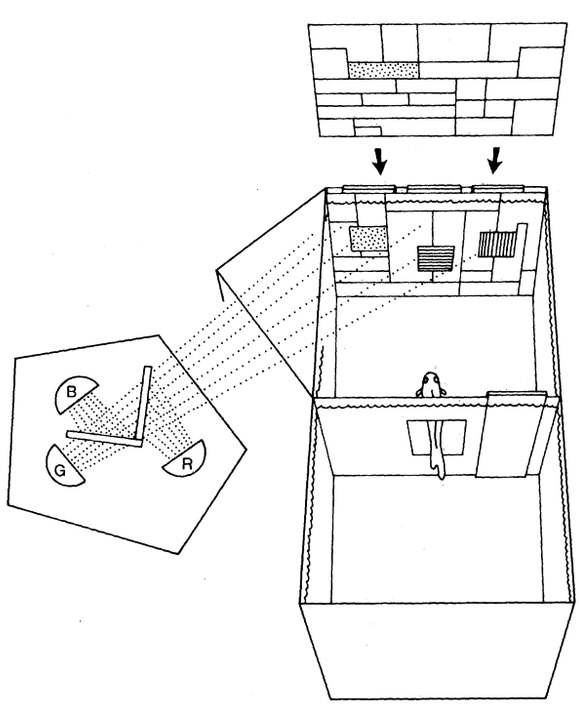

Fig. 1. Goldfish were tested in a 10-gallon aquarium with a water-filled prism attached to the left side, through which light was projected (*8*). In an otherwise dark room, this light was reflected from a 28-cm^2 stimulus insert immersed at one end of the aquarium. During early training, colored papers were placed behind each aperture in a white insert. Next, the fish viewed the aperture colors within a Mondrian insert (shown here within the tank), and finally the fish confronted Mondrian inserts without apertures (shown above). The target color is identified by stippling.

plus yellow-green, (ii) yellow-green plus orange, or (iii) orange plus red. Whereas the random choice score would thus be only 33 percent correct, the three fish performed almost perfectly in condition 1 (Table 1). The fish trained to green selected green, and the other two animals, trained to yellow, selected yellow. Each correct choice elicited a bit of liver delivered from behind the Mondrian once the fish had thrust its snout into the aperture.

Two additional illumination conditions were used for a critical comparison of fish color perception with human perception. In condition 2, the relative intensities of all three lights varied so that the light reflected from the positive patch (either green or yellow) was equal for all three wavelengths, with the consequence that fish A would confront a green paper whose spectral distribution of reflected light was identical to that of the gray paper in condition 1. On alternate test days, fish A was exposed to condition 3, in which the green patch had the same spectral composition as the yellow paper had in condition 1. Similarly, fish B and C confronted in condition 2 a yellow paper whose reflected light had the same spectral composition as that from the gray paper under condition 1. Under condition 3 this yellow paper reflected the same light as the green paper had under condition 1. If the local wavelength composition alone determined the color, fish A would perceive the training color (green) as gray under condition 2 and as yellow under condition 3. Fish B and C would perceive the training color (yellow) as gray under condition 2 and as green under condition 3. To diminish the possibility that fish were identifying the positive color by its association with particular surrounding colors, we alternated two Mondrians with different arrangements of colors around the three apertures. Despite these variations, all three fish continued with 90 to 100 percent correct scores under conditions 2 and 3 (Table 1). Although fish were reinforced for all responses under these two test conditions, good discrimination was maintained by interspersing training condition 1 between the generalization test sessions.

The abilities of these fish to select a target on the basis of color when size, shape, and location on the Mondrian were made more variable was next tested with two flat Mondrians (without apertures) in which the target was a green or yellow rectangle placed in one quadrant (see cover). The location and orientation of the target color was varied by rotating these Mondrians by 90° or 180° from trial to trial: that is, it was sometimes a vertical and sometimes a horizontal rectangle. For each Mondrian the target color was surrounded by a different set of colors to eliminate contextual cues. Fish A and B generalized their experience with the apertures immediately to these new Mondrians even though the reward had to be delivered from in front of the Mondrian after the fish pecked at the correct color. The choice behavior was unambiguous: fish either directly approached a given color and mouthed it, or they circled in front of the Mondrian and returned to the start compartment for another run. Since generalization scores were 96 and 100 percent correct for these two fish in condition 1, daily tests continued to alternate with conditions 2 and 3, as before. Each fish performed with only one or two errors out of 25 further tests trials on each condition (Table 1).

Table 1. Performance scores of three goldfish on two critical color discrimination tests. The number of correct trials per total test trials is given for each lighting condition. Only behavior under condition 1 was differentially reinforced. Statistical evaluation was deemed unnecessary with such consistently low error rates.

Fish	Condition		
	1	2	3
	Mondrian with apertures		
A	30/30	19/22	23/24
B	23/23	19/20	20/20
C	42/46	20/20	21/24
	Flat Mondrians A and B		
A	25/25	25/25	24/25
B	24/25	24/25	23/25
C	24/30	(retrained)	
	20/21	23/25	24/25

Fish C initially scored only 18 correct trials out of 27 with the flat Mondrians under condition 1, choosing yellow-green instead of yellow on many trials. Since this fish did not seem to survey the whole array, retraining this fish was done with more familiar tasks: flat Mondrians with four larger colored squares matched to the aperture colors of the first experiment but presented at two rotations. Since errors ceased after a few training days, flat Mondrians A and B were followed by conditions 2 and 3. Now fish C performed as well as fish A and B on the critical generalization tests (Table 1).

The experiments indicate that goldfish determine the color of an object, as human observers may, within the context of the background array, rather than by the spectral composition of light reflected from the object. This demonstration is not subject to the criticism (as reports of colors by human observers might have been) that selection might reflect subjective impressions previously conditioned or suggested by the context of testing. It is hard to imagine that such a cognitive bias can guide the fish toward its target color during these very different illumination conditions. This laboratory demonstration confirms ecological speculations, based on underwater photometry, that color identification by fish must be achieved under a wide variety of natural lighting conditions (*11*). In addition, fish have evidently evolved this ability without having an identifiable neocortex. A subcortical visual mechanism may thus exist in the fish to compute color relations over wide areas of the visual field (*12*). Although the goldfish retina contains many double-opponent ganglion cells, which may encode relationships between nearby colored areas (*13*) and constitute a part of this mechanism, it seems likely that the final computation of color takes place at central visual stations in the midbrain or diencephalon. Further physiological study of fish should help to identify the anatomical basis of the retinex computations, and comparative studies of color coding by cortical neurons may also help to specify facets of color vision for which primates gain special advantage over lower vertebrate species.

Note added in proof: We have now trained five additional goldfish, including two with ablation of the telencephalon, to select as targets red-orange or blue colors. All fish show color constancy when tested as described in this report.

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9. The Mondrians were made by affixing Color-Aid papers directly to sandblasted aluminum sheets (28 cm^2) by means of 3-M photomount spray adhesive. Each Mondrian contained 23 colored papers cut as squares or rectangles (sometimes partly superimposed), including the primary spectral colors plus violet, brown, white, black, and gray (Fig. 1). Each Mondrian insert was waterproofed by spraying first Photomount and

then McDonald's Protect-A-Coat lacquer on the surface, which made the insert serviceable for about 6 weeks.
10. Three separate lights were projected through band-pass interference filters (450 nm, 545 nm, and 690 nm) and, to eliminate colored shadows, superimposed by two-way mirrors to form a common projection beam (Fig. 1). The beam passed normal to the face of a water prism onto the entire Mondrian at a 45° incident angle. White plastic inserts on the aquarium floor and opposite wall prevented undue specular reflections. Before each test session, the relative intensities of the long-, middle-, and short-wave light reflected from the colored target paper in the Mondrian and other Mondrian papers relatively near in hue were measured with a telephotometer (Spectra Pritchard, 1980A).
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Heat Generated by the Dark-Adapted Squid Retina in Response to Light Pulses

Abstract. *A rapid increase in the temperature of the dark-adapted squid retina evoked by a brief light pulse was detected with a pyroelectric detector. The amount of heat generated by the retina in response to a pulse of blue light of moderate intensity was far greater than that produced by direct conversion of the stimulating light by the retinal pigments into thermal energy.* D-*Glucose in the medium was required to maintain the ability of the retina to produce light-evoked thermal responses.*

In a variety of invertebrate eyes, readily detectable electrical responses can be evoked by a very small number of photons absorbed by visual pigments (*1*). It is generally believed that the photochemical reaction initiated by absorption of light by visual pigments is followed by a succession of biochemical reactions that amplify the effect of light stimulation and eventually lead to the generation of electrical responses (*2*). How this amplification takes place is not precisely known.

We report here that exposure to a brief light pulse evokes a rapid increase in the temperature of the dark-adapted squid retina and that the amount of heat generated is far greater than that associated with the stimulating pulse itself. We also present evidence in support of the view (*3*) that the process of energy transduction in the retina is dependent on the oxidation of sugar.

To detect heat production we used a pyroelectric detector constructed with poly(vinylidene fluoride) (PVDF) film (Kureha Chemical). PVDF is a synthetic polymer that becomes highly pyroelectric when heated and stretched in the presence of a high electrical field (*4*). The film we used was about 9 μm thick and had a 10-nm layer of aluminum deposited on each surface; a 1°C change in the temperature of this film produces a change of about 5×10^{-9} C/cm^2 in the electrical charge on the aluminum layer. Radiant heat of about 1 μsec in duration has been detected by PVDF film (*5*). We have measured the heat generated by the crab nerve during excitation with a time resolution of about 1 msec (*6*).

A piece of PVDF film about 0.4 by 1 cm^2 in area was glued to a thin (25-μm) platinum plate and was attached to a 4-μm-thick sheet of Mylar; it was connected to an operational amplifier (Analog Devices model 515) with a high (2×10^9 ohm) load resistance and a small (about 3×10^{-12} farad) capacitor (Fig. 1). The output of the operational amplifier was led to a signal averager (Nicolet Instrument model 1072) through an a-c–coupled amplifier with a gain of 200.

The magnitude of the signal recorded under these conditions is proportional to the rate of change of the temperature of the film (*7*). When the heat capacity of the sample under study is far larger than that of the temperature-sensitive portion of the detector, the rate of increase in the temperature of the sample is given by V/apR), where V is voltage at the output of the operational amplifier, a is the area of the PVDF film (in square centimeters), p is the pyroelectric coefficient of the PVDF film (in coulombs per square centimeter per degree Celsius), and R is the load resistance (in ohms). The validity of the relation was substantiated by measuring the heat generated by pulses of green light of known intensities absorbed by a slice of 2 percent gelatin gel that was stained with a mixture of chlorphenol red and phenol red and that absorbed 95 percent of the transmitted light. The time resolution of the detector, determined primarily by the time constant of the operational amplifier, was approximately 6 msec.

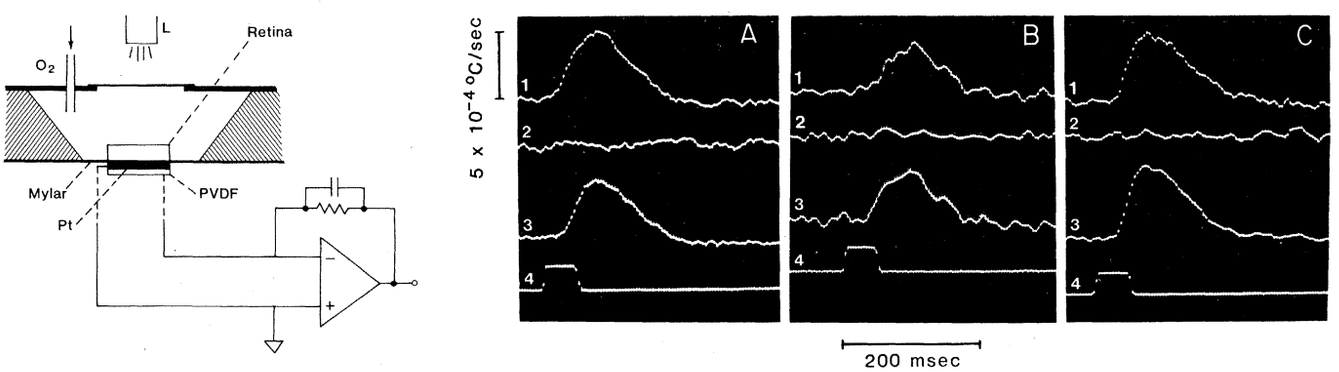

Fig. 1 (left). Schematic diagram of the experimental setup used for measuring the rate of increase in the temperature of the retina caused by stimulating light pulses. L represents a bundle of optical fibers for guiding light from an incandescent lamp; Pt, a thin platinum plate; and PVDF, a sheet of poly(vinylidene fluoride). The potential difference across PVDF film was recorded with an operational amplifier. Platinum was inserted to ensure a spatially uniform increase in the temperature of the PVDF film and to prevent possible mechanical changes in the retina from affecting the PVDF film. Fig. 2 (right). Records showing the temperature increase (expressed in degrees centigrade per second) of the dark-adapted squid retina evoked by light pulses (indicated by traces 4). The light used was 500 nm in wavelength, 25 μW/cm^2 in intensity, and 50 msec in duration. Trace 2 in (A) was taken 10 minutes after switching oxygen to nitrogen; trace 3, 7 minutes after the resumption of oxygen flow. Trace 2 in (B) was taken 40 minutes after immersion of the retina in artificial seawater free of D-glucose; trace 3, 10 minutes after addition of D-glucose (2 m*M*) to the medium. Trace 2 in (C) was obtained 18 minutes after application of 1 m*M* sodium azide; trace 3, 12 minutes after removal of azide. All the records were taken after averaging the signals over 16 trials repeated at 9-second intervals at 20°C.