ondary structures of 5S ribosomal RNA (rRNA) from *Sulfolobus*, and to a lesser degree from *Thermoplasma*, while related to the eukaryotic 5S pattern, differ more from those of eubacteria, halobacteria, and methanogens than do even eukaryotic 5S rRNA (*9*). The initiator tRNA's of sulfur-dependent bacteria also show this pattern; for example, the sequence of the initiator tRNA of *Halococcus* more closely resembles those of eubacteria than of eukaryotes while the sequence of *Sulfolobus* initiator tRNA is closest to that of *Saccharomyces*, and that of *Thermoplasma* is intermediate (*10*). Significant amounts of long polyadenylated sequences are found in *Sulfolobus* RNA and are similar to those in eukaryotic messenger RNA's (mRNA), whereas much lower amounts (30 times less) are found in eubacteria (*11*).

Only a small number of sulfur-dependent archaebacteria have been isolated so far; however, both anaerobic and aerobic taxa are known. Many of them are capable of a novel anaerobic, purely chemolithoautotrophic metabolism utilizing H_2, CO_2, and elemental sulfur as a terminal electron acceptor (*12*). Hence, they prominently occupy unusual niches.

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References and Notes

1. O. Kandler, Ed. *Archaebacteria* (Fischer, New York, 1982).
2. J. A. Lake, E. Henderson, M. W. Clark, A. T. Matheson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5948 (1982).
3. It has been proposed (J. A. Lake, M. W. Clark, E. Henderson, M. Oakes, *Proc. Natl. Acad. Sci. U.S.A*, in press) that the sulfur-dependent bacteria constitute a group (the eocytes) with a phylogenetic importance equal to that of the eubacteria, archaebacteria, and eukaryotes. It has been further proposed that, when this phylogenetic interpretation is confirmed by subsequent additional data, eocytes should be given kingdom status and the name Eocyta (dawn + cell).
4. W. Zillig, R. Schnabel, K. O. Stetter, *Curr. Topics Microbiol.*, in press.
5. K. Stetter and W. Zillig, in *The Bacteria*, C. R. Woese and R. Wolfe, Eds. (Academic Press, New York, in press), vol. 8.
6. J. A. Lake, *Sci. Am.* **245**, 84 (August 1981).
7. J. Huet, R. Schnabel, A. Sentenac, W. Zillig, *EMBO J.* **2**, 1291 (1983).
8. C. Woese, B. Kaine, R. Gupta, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3309 (1983).
9. G. E. Fox, K. R. Luehrsen, C. R. Woese, in (*1*), pp. 330–345.
10. Y. Kuchino, M. Hiara, Y. Yabusaki, S. Nishimura, *Nature (London)* **298**, 684 (1982).
11. M. Ohba and T. Oshima, in (*1*), p. 353.
12. F. Fischer, W. Zillig, O. Stetter, G. Schreiber, *Nature (London)* **301**, 511 (1983).
13. We thank J. Washizaki for electron microscopy and photographic assistance; R. Dickerson, D. Eisenberg, W. Fitch, E. Smith, and A. Klug for discussions; and J. William Schopf for suggesting the name "eocytes." This work was supported by research grants from the National Science Foundation (PCM 76-14710 to J.A.L.), the National Institute of General Medical Science (GM 24034 to J.A.L.), and the Natural Sciences and Engineering Research Council of Canada (A6546 to A.T.M.).

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Far Red Bioluminescence from Two Deep-Sea Fishes

Abstract. *Spectral measurements of red bioluminescence were obtained from the deep-sea stomiatoid fishes* Aristostomias scintillans *(Gilbert) and* Malacosteus niger *(Ayres). Red luminescence from suborbital light organs extends to the near infrared, with peak emission at approximately 705 nanometers in the far red. These fishes also have postorbital light organs that emit blue luminescence with maxima between 470 and 480 nanometers. The red bioluminescence may be due to an energy transfer system and wavelength-selective filtering.*

Emission spectra of luminescent marine organisms generally occur in the blue and green spectral regions (*1, 2*). However, red luminescent flashes have been observed, but never measured, in the deep-sea stomiatoid fishes *Aristostomias scintillans* (*3*) and *Pachystomias* spp. (*4*). The red light originates from suborbital light organs, which fluoresce red in ultraviolet light (*5*). The suborbital light organs of another stomiatoid, *Malacosteus niger*, also contain red-fluorescent material, although no visual observations of red luminescence have been reported for this species. We now present emission spectra from suborbital light organs of *Aristostomias scintillans* and *Malacosteus niger*.

Spectra were recorded at sea on freshly trawled specimens with the use of a computer-controlled, intensified optical multichannel analyzer (OMA). Operation, collection optics, calibration, and correction and data analysis procedures for this system have been described (*1, 6*). Specimens were caught by opening-closing midwater trawls with thermally insulated cod ends (*7*).

No light was visible from the suborbital light organs of the first specimen of *Aristostomias scintillans*. When norepinephrine ($10^{-4}M$) was applied topically

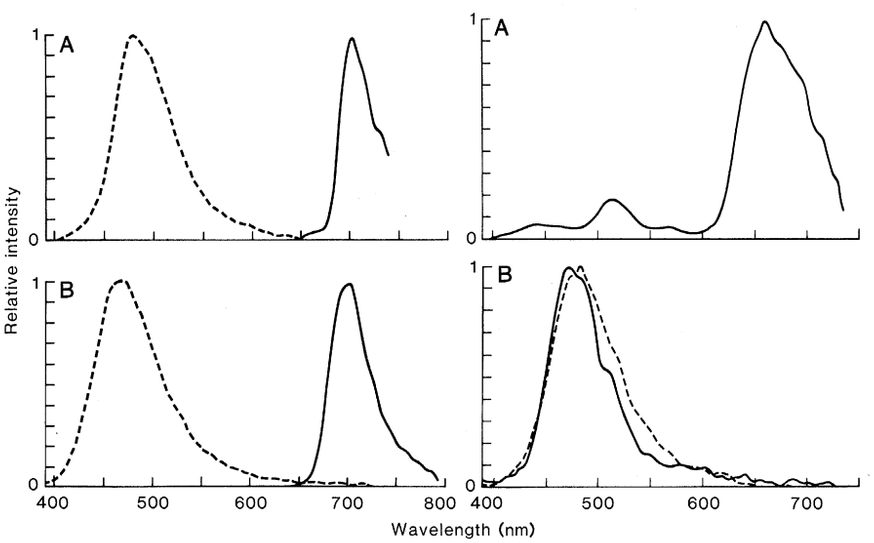

Fig. 1 (left). (A) Emission spectra from a specimen of *Aristostomias scintillans*, fiber-optic input. Each curve represents standardized relative intensity with respect to wavelength. (- - -) Postorbital light organ: λ_{max}, 479 nm; FWHM, 67 nm; signal to noise ratio, 82. (——) Suborbital light organ: λ_{max}, 703 nm; FWHM, 47 nm; signal to noise ratio, 39. The spectrum ends at 750 nm, the long wavelength polychromator limit used for this specimen. (B) Emission spectra from *Malacosteus niger*, double lens collecting optics. (- - -) Postorbital light organ: λ_{max}, 469 nm; FWHM, 77 nm; signal to noise ratio, 87. (——) Suborbital light organ: λ_{max}, 702 nm; FWHM, 49 nm; signal to noise ratio, 94. Fig. 2 (right). Emission spectra measured from *Malacosteus niger*, double lens optics. (A) Suborbital light organ after superficial tissue was removed and organ was cut open: λ_{max}, 660 nm; FWHM, 76 nm; signal to noise ratio, 86. (B) Postorbital light organ. (——) Before isolation: λ_{max}, 471 nm; FWHM, 65 nm; signal to noise ratio, 44. (- - -) After isolation: λ_{max}, 483 nm; FWHM, 74 nm; signal to noise ratio, 34.

to the organs, red light was measured from about 650 to 690 nm, the long wavelength limit of the polychromator at that time. For studies of the next specimen the polychromator spectral window was extended to 750 nm, and a wavelength of maximum emission (λ_{max}) at 703 nm [full width at half-maximum (FWHM) of 47 nm] was observed although the spectrum of the light from the organs extended beyond 750 nm (Fig. 1A). Mechanically stimulated red flashes were visible to the dark-adapted human observer. When mechanical stimulation no longer produced red flashes, norepinephrine was applied topically to the light organ. This resulted in a glow which was visible only with a red-sensitive image intensifier (model 221, Javelin Electronics) but which was still detectable by the OMA. Spectra of this glow did not differ from those associated with mechanically stimulated flashes. The postorbital light organ emitted blue flashes (λ_{max} at 479 nm, FWHM of 67 nm) (Fig. 1A) that were synchronized with the red flashes from the suborbital light organs during mechanical stimulation.

Blue flashes from the postorbital light organs of *Malacosteus* had a λ_{max} of 471 ± 1 nm (mean ± standard deviation) and a FWHM of 71 ± 6 nm (signal to noise ratio was 61 ± 19, $n = 4$ measurements); this result is similar to those reported earlier (*2*). Although no light was visible from the suborbital light organs of *Malacosteus*, measured emissions closely matched those from *Aristostomias*. The mean emission maximum was at 705 ± 2 nm, and the FWHM was 46 ± 3 nm (signal to noise ratio was 53 ± 22, $n = 6$) (Fig. 1B). When the spectral window of the polychromator was extended to 795 nm, the average emission intensity of luminescence at this wavelength was 4.5 ± 1.9 percent ($n = 3$) of that at λ_{max}.

The spectral emission of the suborbital light organ of *Malacosteus* was altered by excision of its brown surface layer, which apparently acts as a filter for light of short wavelength. Relative intensities at shorter wavelengths were increased further by cutting open the organ (Fig. 2A). This broadened the red emission (FWHM = 76 nm), shifted the λ_{max} to 660 nm, and revealed a secondary emission peak at 515 nm with a broad, short wavelength shoulder around 450 nm and a long wavelength shoulder at 565 nm. The emission was dim and not recognizably red in color. Isolation of the postorbital organ produced only a slight shift in spectral emission (Fig. 2B).

Although emission spectra from the suborbital light organs of *Aristostomias* and *Malacosteus* are similar, red emission has been observed only in *Aristostomias*. However, these wavelengths are beyond the spectral range of human scotopic vision and are at the very limits of human photopic vision (*8*). Thus, if the emissions from *Malacosteus* suborbital light organs are characteristically dimmer than those from *Aristostomias*, they might easily not be visible since the emissions from *Aristostomias* were apparently near the human visual threshold.

Red bioluminescence is extremely rare. The only other documented example is from the head of the phengodid beetle, *Phrixothrix* (*9–11*). Our measurements appear to be the first available for any marine red luminescent system.

It has been suggested that red luminescence in these fishes could be used to illuminate prey, or for intraspecific communication, without danger of detection by generally red-insensitive, deep-sea animals (*4, 12, 13*). However, such functions would require a high degree of visual sensitivity since far red light is rapidly attenuated by seawater (*14*). Long wavelength sensitivity may be increased in *Malacosteus* by a yellow lens and a red tapetum (*4, 13*) and in *Aristostomias* by visual pigments with absorption maxima at 555 and 526 nm (*3*). Although these maxima are not a close match to the 703-nm emission maximum, perhaps a pigment that absorbs light of longer wavelength was bleached since the retinal dissections were performed under "a deep red light (Kodak filter No. 1)" (*15*). The filter tissue of the *Malacosteus* subocular photophore has high absorbance below 700 nm (*16*) and reduces total light emission because, with the filter tissue removed, the emission intensity at 660 nm was more than twice that at 705 nm (Fig. 2A). This suggests that it is more important to filter out light of short wavelengths than to increase the intensity of the emission.

The multiple emission maxima of the isolated suborbital light organ of *Malacosteus* suggest that the long wavelength emission could result from an energy transfer system analogous to that found in some coelenterates (*17*). The presence of a green-fluorescent protein (GFP) in pennatulids increases the quantum yield and shifts the spectral emission of the luciferin-luciferase reaction by accepting energy from oxidized luciferin and emitting it as light with the same spectral distribution as the fluorescence emission of GFP. The orange-red fluorescent material found in suborbital light organ extracts of *Malacosteus niger* and *Aristostomias* spp. has been suggested as a possible secondary emitter (*2*). The fluorescence emission maxima of 516, 565, and 635 nm that were measured for *Malacosteus* (*2*) are similar to the bioluminescent maxima emitted from the interior of the suborbital light organ. In the postorbital organs, where secondary emitters are absent, the luciferin-luciferase reaction produces blue light. It seems likely, however, that in the suborbital organs the energy is transferred to fluorescent emitters, which emit at longer wavelengths and possibly with greater efficiency. Such a mechanism in *Malacosteus* and *Aristostomias* differs from the *Phrixothrix* red luminescent system, which involves neither energy transfer nor filtering (*10, 11*).

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References and Notes

1. E. A. Widder, M. I. Latz, J. F. Case, *Biol. Bull.* **165**, 791 (1983).
2. P. J. Herring, *Proc. R. Soc. London Ser. B* **220**, 183 (1983).
3. W. T. O'Day and H. R. Fernandez, *Vision Res.* **14**, 545 (1974).
4. E. J. Denton, J. B. Gilpin-Brown, P. G. Wright, *J. Physiol.* **208**, 72P (1970).
5. P. J. Herring, in *Bioluminescence and Chemiluminescence*, M. A. DeLuca and W. D. McElroy, Eds. (Academic Press, New York, 1981), pp. 527–530.
6. Measurements were made with a 1-mm entrance slit and 20-second integration times. For multiple measurements, λ_{max} and FWHM are expressed as the mean ± standard deviation. Measurements with signal to noise ratios below 30 were not included.
7. Two specimens of *A. scintillans* were collected during July 1983 by the R.V. *New Horizon* off the southwest coast of Oahu, Hawaii; five *M. niger* specimens were obtained on cruise 140 of the R.R.S. *Discovery* off the northwest coast of Africa.
8. The relative luminous efficiency of radiant energy at 700 nm is 0.41 percent of that at 555 nm, the peak of spectral sensitivity for human photopic vision [A. Stimson, *Photometry and Radiometry for Engineers* (Wiley, New York, 1974).]
9. D. L. Tiemann, *Natl. Geogr. Mag.* **138** (No. 1), 56 (1970).
10. W. D. McElroy, H. H. Seliger, M. DeLuca, in *The Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1974), vol. 2, pp. 411–460.
11. E. N. Harvey, *Bioluminescence* (Academic Press, New York, 1952).
12. H. R. C. Fernandez, *Vision Res.* **19**, 589 (1978).
13. H. Somiya, *Proc. R. Soc. London Ser. B* **215**, 481 (1982).
14. Blue light (470 nm) is attenuated by 2 percent, far red light (700 nm) by 48 percent, and near infrared light (760 nm) by 92 percent per meter of clear ocean water [J. N. Lythgoe, in *Handbook of Sensory Physiology*, H. J. Dartnall, Ed. (Springer-Verlag, Berlin, 1972), vol. 7, pp. 566–603; R. C. Smith and K. S. Baker, *Appl. Opt.* **20**, 177 (1981)].
15. Kodak Filter No. 1 has a transmittance of 10 percent for wavelengths longer than 690 nm and

drops to 1 percent transmittance only at 620 nm [Kodak Technical Publication No. B-3H, *Kodak Filters for Scientific and Technical Uses* (Eastman Kodak, Rochester, N.Y., 1972).

16. E. J. Denton and P. J. Herring, unpublished data.
17. J. G. Morin and J. W. Hastings, *J. Cell. Physiol.* **77**, 313 (1971); J. E. Wampler, Y. D. Karkhanis, J. G. Morin, M. J. Cormier, *Biochim. Biophys. Acta* **314**, 104 (1973); W. W. Ward and M. J. Cormier, *Photochem. Photobiol.* **27**, 389 (1978).
18. For assistance at sea we thank J. Childress, T. Frank, and the captains and crews of the R.V. *New Horizon* and R.R.S. *Discovery*. We thank T. Bailey for taxonomic assistance and P. Hiller-Adams for critical comments. Supported by grant N00014-75-C-0242 from the Office of Naval Research, the FBN Fund, and faculty research funds from the University of California, Santa Barbara. Work at sea was supported in part by grant OCE 81-10154 to J. J. Childress from the National Science Foundation.

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Inhibition of Cholesterol Crystal Formation by Apolipoproteins in Supersaturated Model Bile

Abstract. *Apolipoproteins A-1 and A-2 were purified from human plasma. At concentrations present in human bile these proteins prolonged the nucleation time of cholesterol monohydrate crystals when added to model systems of supersaturated bile. In contrast, apolipoprotein C-3 and other serum proteins did not have this effect. Also, when human gallbladder bile was fractionated by gel filtration chromatography, apolipoproteins A-1 and A-2 were among the proteins present in a fraction of bile enriched in potent inhibitors of cholesterol crystal nucleation. These findings suggest that apolipoproteins A-1 and A-2 in supersaturated human gallbladder bile could inhibit the rate of formation of solid cholesterol crystals and thus help to prevent spontaneous cholesterol gallstone formation in humans.*

A critical step in the formation of cholesterol gallstones is the nucleation of excess cholesterol from supersaturated human gallbladder bile. Supersaturated bile, however, does not necessarily lead to the formation of cholesterol crystals or gallstones; for example, more than 50 percent of subjects without gallstones have bile supersaturated with cholesterol (*1, 2*). Earlier we showed that supersaturated bile from gallstone patients differs from that of patients without stones. Nucleation time (the time required for earliest detection of cholesterol monohydrate crystals in an isotropic solution) of supersaturated bile from patients without gallstones was consistently prolonged when compared with bile from patients with cholesterol gallstones (*3, 4*).

Studies conducted in our laboratory (*5*) have provided evidence that biliary proteins contain components capable of inhibiting cholesterol crystal nucleation in human gallbladder bile. Cholesterol crystals were found to form (i) more slowly in supersaturated gallbladder bile from subjects without gallstones than in artificial bile solutions of identical lipid compositon; (ii) more rapidly in supersaturated gallbladder bile from subjects without gallstones after removal of proteins than in the same bile before removal of proteins; and (iii) more slowly in artificial bile solutions after addition of a delipidated, crude protein fraction isolated from human gallbladder bile, an effect that is abolished by Pronase treatment. Further, two protein fractions of gallbladder biles were obtained by molecular sieve chromatography. The first protein fraction eluted before the biliary lipids, and the second protein fraction eluted along with the biliary lipids. This second protein fraction had a more potent effect on cholesterol crystal nucleation inhibition than the first fraction. These data suggest that one or more proteins are present primarily in the second protein fraction of human gallbladder bile, which has been shown to inhibit cholesterol crystal formation. The identity of these putative inhibitors of nucleation remains unknown.

We have also described the presence and amount of apolipoproteins (apo) A-1, A-2, C-2, C-3, and B in human gallbladder and hepatic bile and have presented data indicating that apo A-1 and apo A-2 are present in bile as intact polypeptides (*6*). We suggested that biliary apolipoproteins may be involved in the solubilization of biliary cholesterol and phospholipid in a manner analogous to their functions in plasma, possibly as a supplementary system to bile salts and lecithin (*6, 7*). These data suggested a possible role for apo A-1 and apo A-2, the major protein components of high-density lipoproteins, in gallstone pathogenesis.

In view of these observations, we proposed that the protein components in supersaturated bile responsible for inhibition of cholesterol crystal nucleation include one or another of the apolipoproteins. Moreover, if this hypothesis were correct, these apolipoproteins would be expected to occur primarily in the second fraction.

To examine whether apolipoproteins can inhibit nucleation, we studied the effects of purified serum proteins, including apo A-1 and apo A-2, on the nucleation time of cholesterol crystals in model systems of supersaturated bile (cholesterol saturation index, 1.07 to 1.19; total lipid, 200 g/liter; ratio of phospholipid to bile salt, 0.25) (*8*). Some

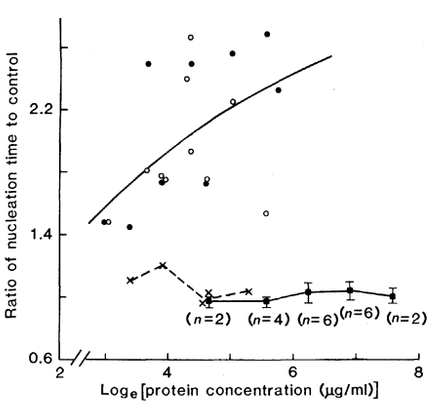

Fig. 1. Effect of apo A-1 (20 to 320 μg/ml), apo A-2 (20 to 250 μg/ml), apo C-3 (30 to 200 μg/ml), and human albumin (100 to 2000 μg/ml) on nucleation time in supersaturated model biles. Data for apo A-1 and apo A-2 were analyzed by a nonlinear regression equation for a rectangular hyperbola: $y = x(1.288 + 0.195)^{-1}$ (standard deviation of regression = 0.373), where y is the ratio of nucleation time to control and x is $\log_e$ (protein concentration). n is the number of observations with albumin; each point for apo C-3 represents a single observation. (●) apo A-1; (○) apo A-2; (x) apo C-3; (■) human albumin.

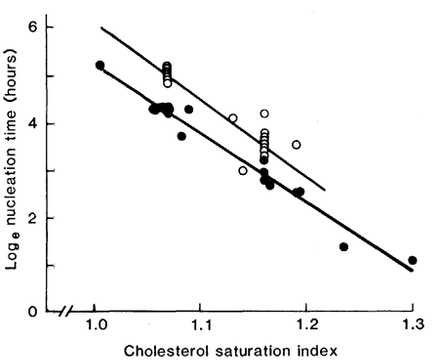

Fig. 2. $\log_e$ (nucleation time) as a function of cholesterol saturation index for a series of model biles without added protein (controls) and for the model biles recombined with apo A-1 and apo A-2. The linear regression equation ($y = ax + b$) for controls is $y = (-14.70 \pm 0.64)x + (19.98 \pm 0.72)$ ($n = 19$, $P < 0.001$; t-test for slope) and for recombinants is $y = (-16.8 \pm 2.29)\ x + (23.05 \pm 2.56)$ ($n = 19$, $P < 0.001$; t-test for slope). For Δa (Δ slope), $P = 0.46$ (not significant); for Δb (Δy-intercepts), $P < 0.001$. At various cholesterol saturation indices, the recombinants nevertheless have consistently longer nucleation times when compared to controls. (○) Recombination with apo A-1 and apo A-2; (●) control.