The viral oncogene of Ha-MuSV contains three initiation codons in the same reading frame and can potentially encode proteins p30, p29, and p21. However, the Ha-MuSV–transformed cells produce mainly the p21 protein, rarely show a minor component of p30 protein, and have never expressed the purported p29 (*4, 6, 17, 18*). The first ATG codon of the Ra-v-*ras* is in a large open reading frame of 747 nucleotides, 21 nucleotides larger than the putative p30-encoding sequence of Ha-v-*ras*. Although a second ATG triplet is located at position 787 of Ra-v-*ras*, where homology with p21-like gene begins, we have never isolated a p21 protein from the immunoprecipitates of RaSV-transformed cells (*10*). The translation of p29 therefore must start at the first ATG codon and continue until the end of the protein, indicating that it is not a cleavage product of a larger protein or synthesized as a precursor of a smaller protein.

We have tested the biological activity of several DNA fragments after cleavage of a 6.4-kbp cloned RaSV segment with various enzymes that would cleave within the RaLV long terminal repeat sequence (Kpn I, Sac I, and Xba I). The 6.4-kbp fragment transformed the transfected mouse NIH/3T3 or the rat cells that expressed a p29 protein; the two smaller fragments containing the Ra-v-*ras* gene but not the long terminal repeat sequence did not transform rat or mouse cells. Since Kpn I cleaves within the region of the RaLV long terminal repeat (*18*), these results indicate that the retroviral promoter sequences are required for the efficient transcription and translation of Ra-v-*ras*.

Complete nucleotide sequences of the normal rat c-*ras* genes have not been published. However, the replacement of glycine with arginine in Ha-MuSV, and with serine in Ki-MuSV has been reported to be the major difference between normal and viral oncogenes (*2, 4*). Thus the four new changes within the p21-like sequence of Ra-v-*ras* and the RaSV-specific sequence in the 5′ half of this gene may represent important deviations from the transforming oncogenes of RaSV and Ha-MuSV. This is not surprising because the oncogenes of both Ha-MuSV and Ki-MuSV were transduced by passage of MuLV in normal rats, whereas Ra-v-*ras* was rescued by RaLV from chemically induced tumors (*7*).

After submission of this report, Capon *et al.* (*21*) reported that the T24 human bladder carcinoma and the normal cellular p21 proteins were identical to the viral Ha-v-*ras* proteins at all amino acid residues except three (residues 12, 59, and 122). Two of these three unique amino acids (residues 59 and 122) of the human p21 are identical to those encoded by the p29 protein of Ra-v-*ras* at precisely the same corresponding positions. Although the biological significance of these changes remains to be determined, the Ra-v-*ras*, in this respect, appears to be more closely related to the human gene than to the viral Ha-v-*ras*.

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Chitin in Sea Anemone Shells

Abstract. *Chitin, which is widely distributed among life forms, is well documented in the coelenterate class Hydrozoa and is contained in one member of class Scyphozoa. In class Anthozoa, hard corals synthesize it but soft corals do not. Chitin was identified by infrared spectrophotometry in the trochoid shell of the actinian* Stylobates. *It constitutes 1.7 percent of the shell by weight, the rest probably being protein. The ability of sea anemones to synthesize chitin is thereby confirmed.*

Chitin is a linear polysaccharide similar in many respects to cellulose (*1–3*). Jeuniaux (*1, 4*) concluded that its wide distribution among fungi, plants, protists, and animals is evidence that the capacity to synthesize it evolved early.

Chitin, in the restricted sense, has repeatedly been demonstrated in the perisarcs of both calyptoblastic and gymnoblastic hydroids; it occurs in chondrophore and millepore hydrozoans as well (*1, 2, 4*). Jeuniaux once proposed (*1*) that this feature distinguishes hydrozoans from other coelenterates but abandoned the idea (*4*) when chitin was demonstrated, albeit rarely, in members of other classes. Among scyphozoans, only the podocyst of *Aurelia aurita* is known to contain this substance (*5*).

Chitin is more common among anthozoans, but may be totally absent in some groups of the class; the several species of octocorals that have been analyzed lack it (*1, 6*). The first record of chitin in a nonhydrozoan coelenterate was from the reef-forming coral *Pocillopora damicornis* (*7*). Wainwright (*7*) proposed that this material, which makes up 0.01 to 0.1 percent of the dry coral skeleton by weight, and which was identified by x-ray diffraction and biochemical tests, forms the organic matrix upon which calcification occurs. Initially Jeuniaux (*1*) acknowledged only that the physical and chemical properties of the compound were near to those of chitin, but chitin has since been found in other corals (*6*).

Pelagic anemones of family Minyadidae secrete from their pedal end a "chitinous mass" that keeps them afloat (*8*); a cuticle envelops the column of many species of actinians; sea anemones of the genus *Adamsia* living on gastropod shells inhabited by hermit crabs may extend the shell's lip by secreting what is variously called a cuticle (*9*), "chitine" (*10*), a horny membrane (*10, 11*), or

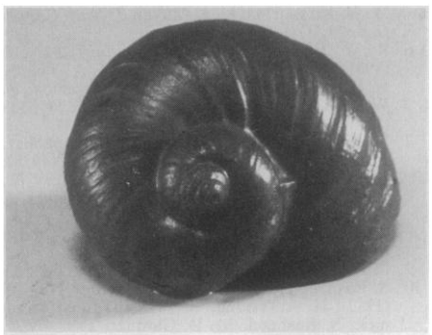

Fig. 1 (left). Shell of *Stylobates aeneus*; greatest width, 60 mm.

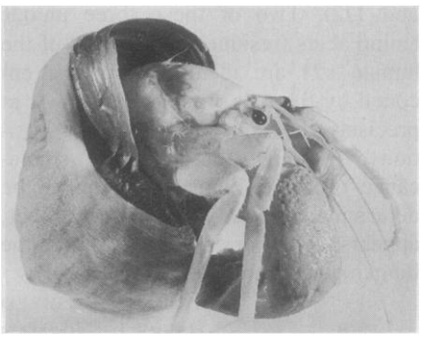

Fig. 2 (right). Anemone shell occupied by hermit crab *Parapagurus dofleini* and enveloped by actinian *Stylobates aeneus* (apex of shell at lower left, anemone oral disk on opposite side; specimen CASIBP 011029).

solidified mucus (*12*). None of these materials has been analyzed chemically. Although Muzzarelli (*3*) reported chitin in *Metridium senile* and Wilfert and Peters (*6*) obtained a positive chitosan test in preserved specimens of *Urticina* (= *Tealia*) *felina*, neither of these anemones secrete ectodermal products and are therefore unlikely sources of chitin. Even though chitin was suggested to be present in the mesoglea of *Edwardsia callimorpha*, it was not found in the only sea anemone examined by Jeuniaux (*1*).

Dunn *et al.* (*13*) discussed the actinian *Stylobates aeneus* Dall, which produces a trochoid "chitinous" shell [(Fig. 1; figure 3 in (*13*)] shaped so much like that of a snail that it was initially considered to be one. Thin and parchment-like in texture, it is secreted by the anemone's pedal disk ectoderm, which is glandular [figure 2 in (*13*)]. More than 100 of these bronze-colored shells, each containing a living hermit crab *Parapagurus dofleini* Balss and covered by the anemone that had produced it (*13*) (Fig. 2), were trapped in Hawaiian seas (*14*).

Before chemical analysis of the shell, its apex, which usually contains remnants of a calcareous shell upon which the anemone first settled (*13*), was removed. Extraction of chitin (*15*) followed ultrasonic washing with dilute (about 1 percent) aqueous sodium dodecyl sulfate, several rinses with deionized water, and drying at 100°C, which caused the shells to become brittle. The product, a fluffy, whitish solid, was washed several times with deionized water and collected on filter paper where it dried to form a thin film. The yield was approximately 1.7 percent by weight.

The infrared absorption spectrum obtained from a small portion of the film (Fig. 3C) agreed closely with that from commercial crab chitin (Fig. 3A) and published spectra (*3*, *16*). Especially characteristic are the amide I band around 1640 cm^{-1} and the amide II band near 1540 cm^{-1}, in addition to the numerous intense bands between 1000 and 1150 cm^{-1} that are typical of cellulose. The infrared spectrum of cleaned, dried, and powdered, but otherwise unprocessed shell (Fig. 3B) approximated that of the extracted material but more closely resembled one typical of protein (*17*). The conventional assay for chitin (*18*), by first converting it to chitosan through incubation in concentrated KOH, which did not dissolve the shell, produced the diagnostic violet color upon addition of 0.2 percent iodine solution, and brown with subsequent exposure to 1 percent H_2SO_4; 75 percent H_2SO_4 then dissolved it. Thus the shell does contain chitin, in the restricted sense.

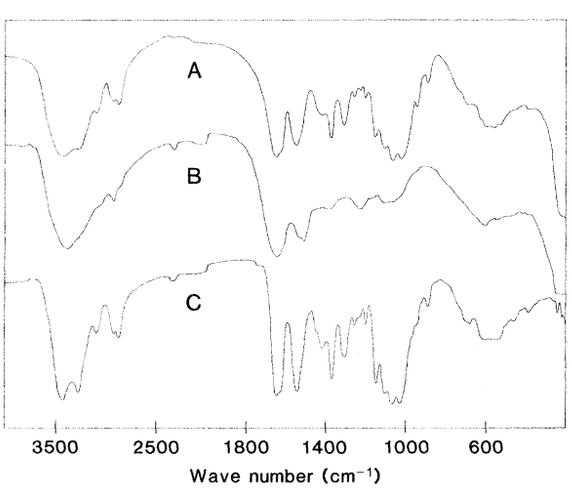

Fig. 3. Infrared absorption spectra (Perkin-Elmer spectrophotometer, model 283). (A) Commercial purified chitin from crab shells (Sigma, No. C-3641) (dispersed in KBr); (B) unprocessed *Stylobates* shell (dispersed in KBr); (C) extracted *Stylobates* shell (unsupported film).

Unprocessed shell ignited at 500°C in a muffle furnace yielded 1.2 percent ash. Spectrographic elemental analysis of the ash showed appreciable amounts of Ca, Si, and Mg, small amounts of Al and Cu, and traces of several other metals. The 98+ percent of shell that is not chitin is probably mainly protein, as judged by the infrared spectrum of the untreated shell and the ash content. Insect cuticles generally contain no more than 25 to 50 percent chitin on a dry weight basis, and some arthropods lack detectable chitin altogether (*2*). *Stylobates* shells exhibit no macroscopic evidence of mineralization. The fact that moist shells are easily dented, much like Ping-Pong balls, suggests that the associated metals may be bound to the protein.

Stylobates is the first sea anemone proved capable of synthesizing chitin and the first anthozoan known to do so in appreciable quantities. Our findings support Jeuniaux's (*1*, *4*) assertion that chitin in coelenterates is synthesized by the ectoderm. *Stylobates* is a member of the largest actinian family, Actiniidae, no other member of which is known to secrete a product resembling chitin. The Minyadidae belong to the same subtribe (Endomyaria) as the Actiniidae, but *Adamsia* and most of the cuticle-forming species belong to another subtribe (Acontiaria) (*6*). Although it is likely that the ability to synthesize chitin is ancient (*1*), these data suggest that that trait may rarely be entirely lost in a lineage. Rather, it is retained in isolated taxa. Perhaps the responsible biochemical pathways are seldom actually lost, so that chitin production is facultatively possible in most species. The sea anemones appear to be an ideal group to analyze for patterns of secretory ability.

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Active Shortening Retards the Decline of the Intracellular Calcium Transient in Mammalian Heart Muscle

Abstract. *When active shortening of the cat papillary muscle was allowed at any time during a contraction, the intracellular concentration of free calcium ions, detected with the calcium-sensitive bioluminescent protein aequorin, was higher than at comparable times in isometric twitches. The difference was not attributable to the differences of length involved or to motion artifacts, and must have been related to the act of shortening or the difference in force development in the two types of contractions. This observation and the phenomenon of shortening deactivation are both consistent with the hypothesis that attachment of cross bridges increases the affinity of the myofilaments for calcium ions.*

It is well known that in many kinds of striated muscle the total duration of contraction is less when the muscle is allowed to shorten during a twitch than when it is constrained to contract isometrically throughout (*1*, *2*). The mechanism of this so-called "shortening deactivation" has not been elucidated, but an obvious possibility is that the intracellular Ca^{2+} transient responsible for excitation-contraction coupling might somehow be abbreviated when shortening is permitted to occur. To test this possibility we used the calcium-sensitive bioluminescent protein aequorin (*3*–*5*) to monitor intracellular calcium transients in the cat papillary muscle—a muscle in which shortening deactivation is prominent under normal conditions (*2*). The results effectively rule out the mechanism just considered and suggest an alternative that is consistent with other evidence.

Aequorin was prepared and microinjected as described previously (*4*). Multiple superficial cells of papillary muscles from the right ventricles of adult cats were injected with the photoprotein; it was usually necessary to inject 30 to 100 cells to obtain satisfactory light signals. After injection, the muscles were mounted vertically between a small clamp (ventricular end) and a 9-0 Tevdek thread (tendinous end) attached to a servo-controlled electromagnetic lever (*6*). A narrow extension of the base of the glass muscle bath (*7*) extended a short distance axially into an ellipsoidal reflector, allowing the muscle to be positioned near one focal point of the reflector; a photomultiplier (EMI 9635A) was mounted so that its photocathode was at the other focal point. This arrangement, which is similar in principle to one illustrated elsewhere (*5*), was designed to optimize light gathering and to minimize any artifacts in the light signal that might result from twisting or rotation of the muscle during shortening. Light (recorded as photomultiplier anode current with time constant 1 msec), force, velocity, and length signals were recorded in analog form on magnetic tape for later analysis. It was usually necessary to average 8 to 64 light signals obtained under identical conditions to obtain a satisfactory signal-to-noise ratio. So that all records would be made under comparable conditions with respect to loading history (*8*, *9*), each test contraction was preceded by seven free-loaded isotonic contractions. Light signals during such contractions did not change detectably during the time required to acquire the signals that were averaged and compared in a given experimental protocol. Except when noted otherwise, experiments were conducted with the initial muscle length set at L_{max} (that is, the muscle length at which tension development was maximal); temperature was 30°C; the stimulus interval was 4 seconds.

Figure 1A shows records made during isometric (a) and free-loaded isotonic (b) contractions starting from the same muscle length. The aequorin signals rise to the same peak level in the two types of

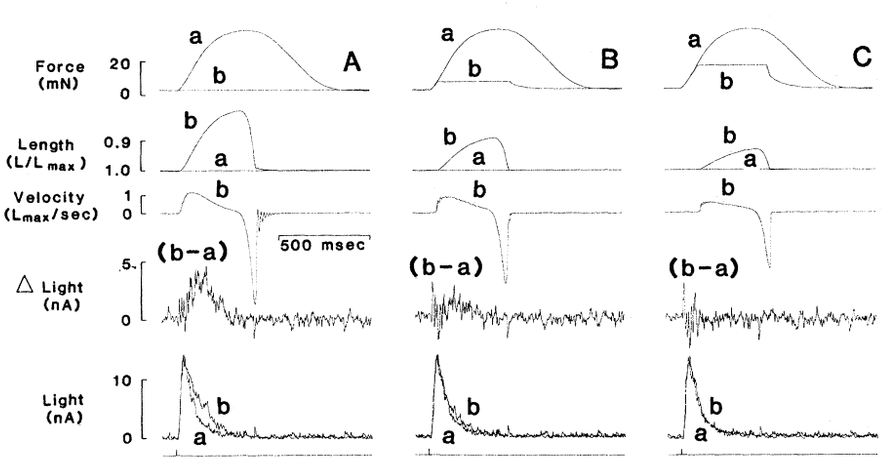

Fig. 1. Aequorin signals of a cat papillary muscle contracting against various loads. Tracings from top downward in all panels: 1, force; 2, shortening; 3, velocity of shortening; 4, light of shortening; 5, aequorin signal; and 6, stimulus. Tracings 1, 2, and 5 show two superimposed twitch contractions—one isometric (a) and one in which shortening occurred (b). The light of shortening is the difference between the two averaged aequorin signals (b − a) obtained by digital electronic subtraction (note that the fourth tracing is displayed with twice the gain of the fifth). Twenty-four signals were averaged. (A) Tracings from an isometric (a) and a free-loaded isotonic contraction (b). The aequorin signals reach the same peak, but the decline of the aequorin signal (Ca^{2+} transient) is initially faster during force development (a) than during shortening (b). The total duration of the light signal is essentially the same in the two contractions. The light of shortening (the difference between the two light signals) becomes apparent some 35 msec after the onset of shortening. (B) The muscle now lifts a light load during the isotonic contraction. The light of shortening is less pronounced. (C) The muscle lifts a heavier load. The light of shortening is practically absent. All tracings in this figure are from the same muscle. Characteristics: length at L_{max}, 5.25 mm; mean cross-sectional area, 0.33 mm^2; ratio of resting to total tension (resting + active) at L_{max}, 0.079.