from the TLC plate, dissolved in 0.1*N* sodium citrate buffer (*p*H 2.2), and confirmed to be L-citrulline by ion exchange chromatography. The intracellular accumulation of L-[^{3}H]citrulline was significantly less when the medium was not supplemented with unlabeled L-arginine or when 0.05 m*M* N^GMMA was added to the medium with 0.4 m*M* unlabeled L-arginine. Stimulated macrophages do not accumulate L-[^{3}H]citrulline intracellularly under the same experimental conditions. These findings demonstrate that, under conditions of culture that support the expression of cytotoxicity (for example, the presence of L-arginine and absence of N^GMMA), increased intracellular L-[^{3}H]citrulline derived from L-[^{3}H]arginine can be detected in CAM.

A pathway oxidizing imino nitrogen derived from L-arginine or L-homoarginine to nitrite (see Fig. 1) has not been described in either prokaryotes or eukaryotes. We propose that arginine deiminase is the first enzyme of this pathway and that it induces multiple metabolic changes in mammalian cells which, in addition to other effects, are capable of controlling cellular proliferation (*16*). It is also possible that this effector system participates in controlling the proliferation of some pathogenic microorganisms, particularly microbes capable of survival in the intracellular environment. The actual mechanism of metabolic inhibition by products of the L-arginine–dependent effector mechanism remains to be defined. However, nitrite or oxygenated nitrogen intermediates in the pathway of nitrite and nitrate synthesis could participate in causing iron loss from aconitase and other enzymes containing Fe-S clusters in target cells of CAM in addition to inducing other intracellular effects.

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The Cytoskeletal Protein Vinculin Contains Transformation-Sensitive, Covalently Bound Lipid

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Vinculin, which is associated with the cytoskeleton of many cells, has been suggested as a possible linker between microfilament bundles and the plasma membrane. Here it will be shown that fatty acid is covalently attached to vinculin in vivo. Furthermore, in chicken embryo fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus, tsNY68, the acylation of vinculin at the permissive temperature was less than one-third that at the nonpermissive temperature. Thus, the covalent binding of lipid to vinculin is a transformation-sensitive event. The covalent modification of vinculin by lipids could be directly or indirectly involved in its reversible association with membranes. This modification may also provide a mechanism to alter the organization of vinculin within cells and thereby play a regulatory role in anchoring or stabilizing microfilament bundles at plasma membranes.

IN CULTURED FIBROBLASTS, MICROFILament bundles terminate at focal adhesion plaques, the specialized membrane regions where cells adhere most tightly to the underlying substratum (*1*). Vinculin is concentrated in these focal adhesion plaques (*2*) and thus may play a role in anchoring or stabilizing microfilament bundles at plasma membranes. Transformation of cells by oncogenic viruses leads to a reduction in the number of focal adhesion plaques and disrupts microfilament bundles concomitantly with markedly altering vinculin organization (*3, 4*). In cells infected with Rous sarcoma virus (RSV), a single gene product, $pp60^{src}$ (a protein kinase that modifies cellular proteins through phosphorylation on tyrosine residues), induces all these alterations (*5, 6*). Vinculin, one of the putative substrates of $pp60^{src}$, shows a marked transformation-dependent increase in phosphotyrosine content (*4*). However, it is believed that vinculin phosphorylation alone is not enough to explain the rearrangement of cytoskeletal elements observed during transformation (*6, 7*).

Recently it has been reported that α-actinin (*8, 9*), protein kinase C (*10*), and a few other proteins (*11*) are functionally regulated by noncovalent association with spe-

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cific lipids such as diacylglycerol or phosphatidylinositol. This suggests that specific, noncovalent interactions of lipids with proteins could control their reversible association with membranes. Covalent attachment of lipids to several viral (*12–15*) and cellular proteins (*15–17*) also occurs. For some proteins, acylation prevents proper intracellular transfer of the proteins (*18*), and recent data by Sefton and colleagues (*19*) show that transformation by pp60^{src} depends on fatty acylation. Thus, regulation of membrane association of vinculin by binding of lipids could be the driving force for assembly and rearrangement of cytoskeletal elements.

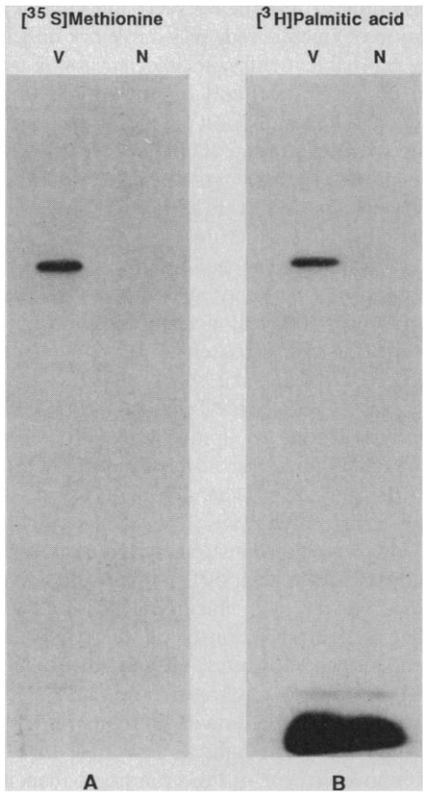

Fig. 1. Vinculin immunoprecipitated from normal CEF contains bound lipid. CEF's were labeled with (**A**) [^{35}S]methionine or (**B**) [^{3}H]palmitic acid for 4 hours. Vinculin was immunoprecipitated from detergent-solubilized cells with antiserum specific for vinculin (V) or with nonimmune serum (N). The immunoprecipitated material was analyzed by SDS-PAGE and processed for fluorography. CEF's were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% tryptose phosphate broth, 2% calf serum, 1% chicken serum, and 1% penicillin-streptomycin. Cells were labeled with [^{35}S]methionine (200 Ci/mmol) or [9,10-^{3}H(*N*)]palmitic acid (30 Ci/mmol, New England Nuclear) by incubation for 4 hours at 37°C. The complete labeling medium consisted of DMEM containing 10% of the normal concentration of methionine, 5 m*M* sodium pyruvate, and [^{35}S]methionine (30 μCi/ml) or [^{3}H]palmitic acid (250 μCi/ml). Cultures were washed three times with cold phosphate-buffered saline (PBS) and were then lysed in 1 ml of cold TNET buffer (50 m*M* tris-HCl, *p*H 7.4, 150 m*M* NaCl, 5 m*M* EDTA, 1% Triton X-100) containing 1 m*M* phenylmethylsulfonyl fluoride (PMSF) and 1% Aprotinin (Sigma A-6279). Lysates were scraped from the plate with a rubber policeman, incubated on ice for 20 minutes, and then clarified by centrifugation for 10 minutes at 15,000*g*. Immunoprecipitations, gel electrophoresis, and fluorography were performed as described by Burn *et al.* (*8*). The [^{35}S]methionine- labeled polypeptide was detected by exposure for 20 hours, and the [^{3}H]palmitic acid–labeled polypeptide was detected by exposure for 20 days to Kodak X-Omat AR-5 film.

To determine whether vinculin contains covalently bound fatty acid, we radiolabeled normal chicken embryo fibroblasts (CEF's) in culture with ^{3}H-labeled palmitic acid, and cellular extracts were immunoprecipitated with antiserum specific for vinculin (antivinculin). A single protein (130,000 M_r) labeled with [^{3}H]palmitic acid that comigrated with [^{35}S]methionine-labeled vinculin was detected in the immunoprecipitates (Fig. 1). Control experiments with nonimmune serum (Fig. 1) or preimmune serum gave no precipitation of labeled compounds. The specificity of the antivinculin was further confirmed by the comigration of purified vinculin with immunoprecipitated protein in one- and two-dimensional gel systems, as well as by immunoblotting techniques. In addition, immunofluorescence studies with the antivinculin or an immunoglobulin G fraction of affinity-purified antibodies resulted in the characteristic staining of focal adhesion plaques.

As previously described (*15, 20–22*), the spectra of proteins labeled with [^{3}H]palmitic acid and [^{35}S]methionine were different, an indication that labeling with [^{3}H]palmitic acid does not represent general labeling of cellular proteins. In addition, labeling with fatty acid was always performed in the presence of 5 m*M* sodium pyruvate and in most experiments for short time periods only (1 to 5 hours), conditions that drastically reduce metabolic conversion of fatty acids into amino acids. Thus, it is likely that the labeling of vinculin by [^{3}H]palmitic acid is the result of fatty acid binding to vinculin and not the conversion of the ^{3}H into amino acids.

These results indicate that palmitic acid, or a derivative of it, may be covalently linked to vinculin since the radiolabel in the immunoprecipitated vinculin (i) was resistant to extensive washing by detergents such as Triton X-100 (TX-100), Nonidet P-40 (NP-40), Empigen BB, and SDS; (ii) was precipitated with vinculin in 10% trichloroacetic acid (TCA); (iii) was not extracted by organic solvents such as chloroform-methanol; and (iv) still comigrated with vinculin after SDS–polyacrylamide gel electrophoresis (SDS-PAGE).

However, to prove that the labeled compound covalently linked to vinculin was

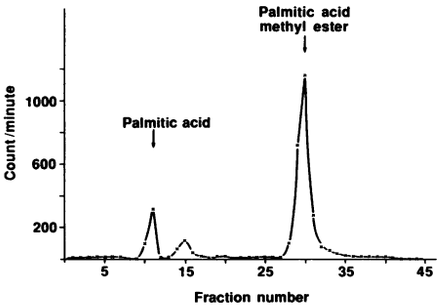

Fig. 2. Reversed-phase high-pressure liquid chromatography (r-HPLC) of lipids covalently bound to vinculin. Vinculin was immunoprecipitated from cells labeled with [^{3}H]palmitic acid, delipidated, and hydrolyzed in methanolic HCl for 16 hours at 90°C (*21*). The released material was analyzed by r-HPLC by using a 4.6 mm by 25 cm Ultrasphere-ODS column. Elution was performed with 80% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. The elution of labeled lipids was compared with the absorbance elution profile of fatty acids and fatty acid methyl esters.

lipid rather than a product of its metabolism into amino acids, we subjected the immunoprecipitated vinculin [after SDS-PAGE (*13*), TCA precipitation, or extensive extraction by organic solvents (*15, 21*)] to hydrolysis in methanolic HCl or methanolic KOH (*15, 21*). Single major radioactive species having the mobility of fatty acid methyl esters were found by high-performance thin-layer chromatography on silica gel. The exact identity of the fatty acid was determined by reversed-phase high-pressure liquid chromatography (r-HPLC) and resulted in the recovery of methyl palmitate (Fig. 2). Thus, palmitic acid was the lipid covalently linked to vinculin. Nevertheless, experiments with [^{3}H]myristic acid indicated that vinculin is also labeled with that fatty acid. This result suggests that there was either an interconversion of myristic acid to palmitic acid (*21*) or an attachment of both fatty acids to vinculin. In the latter case the different fatty acids might even be attached to separate and distinct sites on the same protein. Another alternative might be a covalent attachment of both fatty acids to vinculin by way of phosphatidylinositol as has been described for other cellular proteins (*16*).

Hydroxylamine treatment (*15, 21*) of immunoprecipitated vinculin released only small amounts of radioactive label from [^{3}H]palmitic acid–labeled protein, as was true in control experiments. However, the release of fatty acids from proteins by hydroxylamine is not always quantitative and varies among proteins (*21*). On the other hand, most of the fatty acid was removed from vinculin by treatment with methanolic KOH. Thus, fatty acid, if directly linked to

vinculin, may be bound through an ester bond rather than through an amide linkage.

To determine whether this modification of vinculin is a transformation-sensitive event, we have analyzed the binding of lipid to vinculin in cells infected with a temperature-sensitive (ts) transformation mutant of RSV, tsNY68 (*23*). The advantage of using a ts mutant is that it eliminates the possibility that a change in acylation reflects only a general alteration in fatty acid binding caused by the infection of the cells with a virus. The tsNY68-infected cultures were completely transformed at the permissive temperature (35°C) but were morphologically normal at the nonpermissive temperature (41°C). Cells infected with tsNY68 were labeled for different times (1 to 16 hours) in culture with either [^{3}H]palmitic acid or [^{35}S]methionine. The general ^{3}H–fatty acid labeling of proteins in whole-cell lysates was similar at both the permissive and the nonpermissive temperature as demonstrated by SDS-PAGE and fluorography. These data suggest that general metabolic incorporation and transport of fatty acids do not differ at 35° and 41°C, respectively, in agreement with previous reports (*22*). However, incorporation of ^{3}H–fatty acids into vinculin immunoprecipitated from cellular extracts of tsNY68-infected cells was consistently lower when cells were maintained at the permissive temperature rather than at the nonpermissive temperature (Fig. 3).

Because of the different growth rates of transformed and nontransformed cells and of cells grown at 35° and 41°C, the number of cells and therefore the amount of vinculin in the various cultures differed. Thus, after immunoprecipitation and SDS-PAGE (Fig. 3), the incorporation of [^{3}H]palmitate (54 count/min at 35°C and 502 count/min at 41°C, respectively) and [^{35}S]methionine into vinculin (825 count/min at 35°C and 2295 count/min at 41°C, respectively) was determined. The ratios of [^{3}H]palmitate to [^{35}S]methionine at the permissive temperature (0.066) and at the nonpermissive temperature (0.219) were taken as measures of the relative binding of lipid to vinculin at either temperature. A significant decrease (more than a factor of 3) in the acylation of vinculin occurred at the permissive compared with the nonpermissive temperature. Control experiments with uninfected CEF showed no significant difference in the acylation of vinculin at 35° and 41°C. The same result was found for cells infected with wild-type (Schmidt-Ruppin strain of RSV, subgroup A) virus at either temperature. However, in a series of experiments, the binding of lipid to vinculin in cells infected with wild-type virus was always reduced when compared with noninfected cells, confirming the results obtained with the ts mutant of RSV.

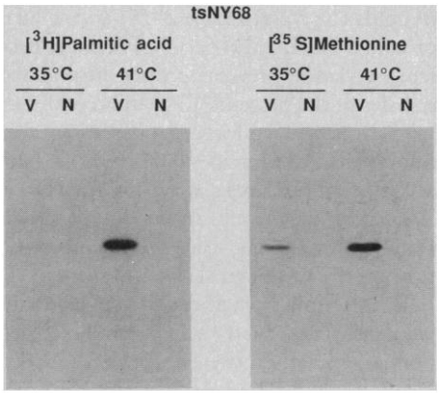

Fig. 3. [^{3}H]Palmitic acid labeling of vinculin is reduced in tsNY68-infected CEF's maintained at the permissive temperature (35°C). CEF's were infected with tsNY68 (*23*) and maintained at the permissive (35°C) or nonpermissive (41°C) temperature. Cells were labeled for 4 hours with either [^{3}H]palmitic acid or [^{35}S]methionine. Labeled proteins were immunoprecipitated with antiserum specific for vinculin (V) or nonimmune serum (N) and subjected to SDS-PAGE. The gel was processed for fluorography and exposed as described in the legend to Fig. 1.

These results show that a reduction of lipid binding to vinculin accompanies transformation of CEF by RSV and correlates with a change in cell morphology. Like others (*22*), we observed no general difference in protein acylation at 35° and 41°C. In addition, recent reports showed that the acylation of pp60^{src} in cells infected with tsNY68 is similar at both temperatures (*24*) or may even be reduced at the nonpermissive temperature (*14*). Thus, all of these results support the conclusion that transformation of CEF by RSV leads to a specific reduction in the acylation of vinculin and is not due to general changes in fatty acid metabolism in transformed cells.

Lipid-binding proteins such as α-actinin (*8*), protein kinase C (*10*), and pp60^{src} (*25*) appear to associate reversibly with plasma membranes. Even though vinculin is not thought to be an integral membrane protein, it may belong to a separate class of proteins that reversibly interact with membranes. Recent results demonstrate specific labeling of vinculin with a photoactivatable analog of lecithin, which selectively labels membrane-embedded protein domains (*26*). It is reasonable to assume that the fatty acid moiety bound to vinculin plays a crucial role in its interaction with membranes. Presumably, only a fraction of the vinculin molecules are modified by fatty acids. Binding of lipid to vinculin may lead to a direct interaction of the protein with the plasma membrane. Alternatively, acylation of vinculin may induce a conformational change in the protein, leading to the exposure of additional hydrophobic sites and thereby regulating its reversible membrane association. Fatty acid acylation has also been implicated in modulating enzyme substrate interactions and protein subunit interactions. Thus acylation of vinculin may play a role not only in its reversible membrane association but also in its localization and organization within cells and focal adhesion plaques. Lipid binding to vinculin may alter the interaction of vinculin with the cytoskeleton and other proteins, as well as its ability to form paracrystalline-like structures (*27*).

Overall, the data presented in this report suggest that the binding of lipid to vinculin could be a powerful mechanism involved in regulating the cytoskeletal rearrangements observed during cell shape changes or in moving tissue culture cells. In addition, an altered binding of lipid to vinculin as observed in virally transformed cells may lead to the phenotypic changes characteristic of the transformed state. The results do not allow us to discriminate between a direct or indirect action of pp60^{src} on vinculin acylation. However, an intriguing possibility is that of a direct action of pp60^{src} on as yet unidentified substrates, such as membrane-bound acylases, acyltransferases, or lipases. Further experiments with mutants of RSV should shed more light on a possible regulatory involvement of lipid binding to vinculin and its postulated function in the maintenance of intact microfilament bundles. Analysis of acylated vinculin in these mutants may further demonstrate a possible connection between acylation and tyrosine phosphorylation of vinculin.

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Fish in Offshore Kelp Forests Affect Recruitment to Intertidal Barnacle Populations

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Kelp forests along the coast of central California harbor juvenile rockfish that prey on the larvae of invertebrates from the rocky intertidal zone. This predation reduces recruitment to barnacle populations to 1/50 of the level in the absence of fish. The dynamics of the intertidal community are thus strongly coupled to the dynamics of the offshore kelp community.

THE EPISODIC RECRUITMENT (THE addition of young to a natural population) of many marine invertebrates (*1*) strongly affects benthic community structure (*2, 3*). The causes of this episodic recruitment are generally unknown, but two components are involved—(i) variation in reproductive output and (ii) variation in the mortality and distribution of larvae while in the water column. This study focuses on the second of these components. We show that substantial numbers of larvae of the intertidal barnacle *Balanus glandula* die as they pass through kelp forests and that yearly variation in the composition of these forests is a major cause of yearly variation in barnacle recruitment.

The giant kelp *Macrocystis pyrifera* is a prominent feature of the Pacific coasts of North and South America (*4*), where it forms continuous beds up to 8 km long and 1 km wide. The extent of these forests and the density of plants within vary greatly in space and time because of storms (*5*), herbivores and their predators (*6*), and major current features (for example, El Niño) (*7*).

A strong negative relation between kelp canopy area and recruitment of *B. glandula* to the rocky intertidal zone was observed during 4 years at Hopkins Marine Station in central California. Annual recruitment to the natural rock substrate varied by two orders of magnitude (*3*); it was high in 1983, low in 1985, and moderate in both 1982 and 1984. Comparable large fluctuations, but of an opposite sign, were seen in the area of the surrounding kelp forest (Fig. 1). The decline in canopy area due to the large winter storms of the 1982–83 El Niño was particularly dramatic. This study examines the potential causal basis for this correlation between kelp area and intertidal recruitment.

Kelp forests interact with the water column and its resident plankton in several ways. (i) The plants exert drag forces that reduce flow velocities and attenuate internal waves (*8*). (ii) They take up nutrients that might be needed for larval growth and survival (*9*). (iii) Kelp forests provide a suitable habitat for larval settlement, thereby depleting larvae from the water before they reach the intertidal habitat (*10*). (iv) Kelp forests harbor invertebrate and piscine planktivores that may further reduce plankton densities (*10, 11*).

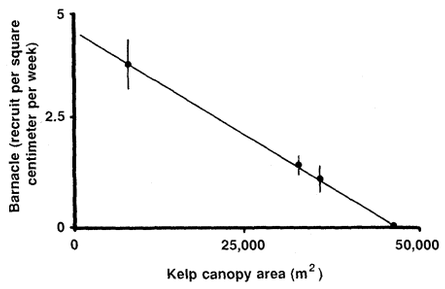

Fig. 1. Correlation of kelp canopy area and *B. glandula* recruitment rates to natural substrate in the rocky intertidal zone. Canopy areas were estimated from infrared aerial photographs provided by ECOSCAN Resource Data of Freedom, California. Recruitment rates were measured from weekly photographs (about 20 per year) of four 35-cm^2 quadrats in the intertidal zone inshore from the kelp forest. Error bars are 95% confidence limits (*22*). Slope = 9.6×10^{-5}; SE = 1.2×10^{-6}.

To examine the effects of kelp beds on the plankton and subsequent inshore recruitment, we collected plankton samples at the inshore and offshore margins of the bed. Three samples (*12*) were collected with a portable pump at fixed stations at approximately weekly intervals beginning in April 1985. *Balanus glandula* releases a nauplius larva. Development proceeds through five additional feeding nauplius stages, terminating in a nonfeeding, cypris larva. Only stage I and stage II nauplii and cyprids were commonly collected close to shore. Both were concentrated in the top meter of the water column (*13*), paralleling their positive phototaxis in laboratory culture (*14*). Therefore, our sampling focused on shallow, surface waters.

Throughout the spring and summer, the plankton samples show steep concentration gradients across the kelp bed (Fig. 2A). *Balanus glandula* cyprids were on average 70 times as abundant in samples collected offshore from the kelp bed as in samples collected inshore. Similarly, a tenfold difference between offshore and inshore concentrations of barnacle nauplii was observed, but the direction of the gradient was opposite to that of cyprids. Comparably steep drops in concentration were consistently found in many other taxa [other barnacle cyprids, copepods, copepod nauplii, gastropod and bivalve veligers, and decapod zoea; Fig. 2A and (*15*)].

The large concentration gradients translate directly into an equally steep gradient in larval recruitment. During July and August of 1985, recruitment was monitored at each of the plankton pumping stations on settling plates affixed to the marker buoys just below the surface (samples, $n = 4$ at each site). On average, recruitment rates by *B. glandula* to these plates were 54 (95% confidence inter-

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