source not available to the urchins and thereby may benefit by minimizing diet overlap with a potential competitor.

Among the macroinvertebrates found at White Point, only the gastropods commonly occur at the vents. Besides the black abalone, the California sea hare (*Aplysia californica*), the giant keyhole limpet (*Megathura crenulata*), and the Norris top snail (*Norrisia norrisii*) graze around the vents. In addition, several species of limpets appear to graze the bacterial mats surrounding intertidal zone vents. A survey of other shallow-water vent sites may provide additional examples of this type of trophic relationship. Ciliates, zooflagellates, and insect larvae have been reported to ingest sulfur-oxidizing bacteria at terrestrial vent systems (*19*), but this is the first account of marine invertebrates consuming this unconventional food source at coastal hydrothermal vents.

JEFFREY L. STEIN*
Department of Biology,
California State University,
Long Beach 90840

References and Notes

1. D. M. Karl, C. O. Wirsen, H. W. Jannasch, *Science* **207**, 1345 (1980). J. H. Tuttle, C. O. Wirsen, H. W. Jannasch, *Mar. Biol.* **73**, 293 (1983).
2. For example, see V. M. V. Vidal *et al.*, *J. Geophys. Res.* **83**, 1757 (1978).
3. R. B. Spies and D. J. DesMarais, *Mar. Biol.* **72**, 67 (1983).
4. J. A. Bland and J. T. Staley, *Arch. Microbiol.* **117**, 79 (1978).
5. The identity of the inclusions as sulfur is supported by (i) their highly refractile (phase-contrast) image and dark peripheral ring when viewed microscopically with bright-field optics, and (ii) their failure to stain with Sudan black B, indicating that they were not poly-β-hydroxybuterate or other lipid storage material. See criteria for identifying bacterial sulfur inclusions by V. B. D. Skerman, G. Dementjeva, and B. J. Carey [*J. Bacteriol.* **73**, 504 (1957)].
6. The sulfide content of the vent effluent was measured according to the titrimetric (iodine) method in *Standard Methods for the Examination of Water and Wastewater* [M. C. Rand, A. E. G. Greenberg, M. J. Taras, Eds. (APHA-AWWA-WPCF, Washington, D.C., ed. 14, 1975), p. 505]. Thiosulfate contamination should be nearly absent because of the low in situ oxygen content of the vent effluent (less than 0.5 ppm).
7. J. L. Campbell, *Trans. Am. Microsc. Soc.* **84**, 376 (1965); G. Owen, in *Physiology of Mollusca*, K. M. Wilbur and C. M. Yonge, Eds. (Academic Press, New York, 1966), vol. 2, pp. 84–86.
8. The percent sulfur was determined gravimetrically [B. Meyer, in *Sulphur, Energy and the Environment* (Elsevier, Amsterdam, 1977), p. 119].
9. A 1-m length of monofilament (20-pound) line was threaded through the ratchet end of a plastic cable-tie that was cemented to the substratum adjacent to an abalone. The line was oriented so that it could be pulled through the cable-tie only in the direction of abalone movement. It was then attached to the abalone's shell at the third or fourth respiratory pore. In this way, the maximum nightly foraging radius could be determined by measuring the length of line pulled through the cable-tie. Foraging distances exceeding 1 m were estimated from the abalone's point of origin to its displaced position the following day (straight line).
10. Epilithic biomass was scraped and suctioned from a 15.3-cm^2 quadrat sampled at intervals along a transect line originating from a vent. Standing crop biomass (measured as ash-free dry weight) declined from 1.3 mg/cm^2, 10 cm from the vent opening, to 0.02 mg/cm^2, 50 cm from the vent opening.
11. M. Bergen, thesis, University of California, Santa Barbara (1971).
12. Juvenile green abalones were obtained from D. Leighton and S. Bent, World Research Inc., San Diego, Calif.
13. D. L. Leighton, M. J. Byhower, J. C. Kelly, G. N. Hooker, D. E. Morse, *J. World Maricult. Soc.* **12**, 170 (1981).
14. Shell growth of juvenile green abalone (20 to 30 mm) averaged 2.41 mm/month when the abalone were raised at 22°C on a diet of macroalgae (*13*). For young adult black abalone in the 40- to 50-mm size class, an average monthly growth of 1.50 mm was obtained for individuals inhabiting the intertidal zone off Santa Cruz Island [M. B. Wright, *Veliger* **18**, 194 (1975)].
15. D. Leighton and R. A. Booloottian, *Ecology* **44**, 2 (1963).
16. H. Kito, S. Kikuchi, N. Uki, in *Proceedings of the International Symposium on Coastal Pacific Marine Life*, C. J. Flora, Ed. (Western Washington University Press, Bellingham, 1980), pp. 55–66.
17. Approximately 150 urchins were placed in and around an active vent (4 m deep). Within 24 hours all urchins directly in the path of the vent effluent were dead or moribund, and others placed within 0.5 m of the vent opening had moved away.
18. H. Theede, A. Ponat, K. Hiroki, C. Schlieper, *Mar. Biol.* **2**, 325 (1969).
19. J. B. Lackey, E. W. Lackey, G. B. Morgan, *Fla. Univ. Eng. Ind. Exp. Stn., Bull. Ser.* **119**, 3 (1965).
20. I thank M. Kleinschmidt and P. Meister for diving assistance and D. McCray for field photography. I thank A. Miller and G. Leister for laboratory space and support, who, along with R. Bray, C. Galt, H. Jannasch, D. Nelson, and D. Leighton, gave helpful comments and criticisms.
* Present address: Vantuna Research Group, Department of Biology, Occidental College, Los Angeles, Calif. 90041.

21 June 1983; accepted 4 January 1984

Somatic Activation of *ras*^{K} Gene in a Human Ovarian Carcinoma

Abstract. *A tumor isolate from a patient with serous cystadenocarcinoma of the ovary contained an activated* ras^K *gene detected by transfection of NIH/3T3 cells. In contrast, DNA from normal cells of the same patient lacked transforming activity, indicating that activation of this transforming gene was the consequence of somatic mutation in the neoplastic cells. The transforming gene product displayed an electrophoretic mobility in sodium dodecyl sulfate–polyacrylamide gels that differed from the mobilities of* ras^K *transforming proteins in other tumors, indicating that a previously undescribed mutation was responsible for activation of* ras^K *in this ovarian carcinoma.*

Transfection experiments with DNA have led to the identification of activated cellular transforming genes in a wide variety of human tumor cell types (*1*). In many cases, these genes are related to the transforming genes of Harvey and Kirsten murine sarcoma viruses, *ras*^{H} and *ras*^{K}. In particular, a human homolog of *ras*^{H} is responsible for the transforming activity of DNA from single human bladder and lung carcinoma cell lines (*2–5*). Activation of a human homolog of *ras*^{K} seems to be more common, however, since this gene accounts for the transforming activity detected in DNA from human carcinoma cell lines of the colon, lung, gallbladder, and urinary bladder and from an acute lymphocytic leukemia cell line (*2, 6–9*). Furthermore, primary tumor isolates of human lung, colon, and pancreatic carcinomas as well as a rhabdomyosarcoma contain an activated form of this gene (*6*). Finally, activation of a third member of the human *ras* gene family, *ras*^{N}, also occurs in many tumor cell types including neuroblastoma, fibrosarcoma, leukemia, and lymphoma cell lines, as well as primary tumor isolates of a colon carcinoma and of an acute myelogenous leukemia (*9, 10*). However, *ras* gene activation has been detected in only about 20 percent of neoplasms, in contrast to other transforming genes, such as *Blym*-1 and *Tlym*-I, which are activated in most (80 to 100 percent) lymphoid tumors of specific cell types (*11, 12*). This suggests that *ras* activation may contribute to the development of neoplasia in a fraction of many types of tumors, but may not be a necessary event for the development of any particular form of cancer.

Ovarian carcinoma is one of the most prevalent forms of cancer in women. Of the many histological subtypes of this disease, the most common is serous cystadenocarcinoma of the ovary, which appears to represent neoplastic transformation of the ovarian surface epithelium. Previous experiments (*6, 13*) in which a limited number of ovarian carcinoma samples were screened failed to detect the presence of activated transforming genes. However, since *ras* activation occurs in only a small percentage of tumors of a given type, we screened DNA from additional samples of ovarian carcinoma for transforming activity in DNA transfection assays.

Ascites fluid was collected from five patients with serous cystadenocarcinoma of the ovary. Morphological examination revealed that these samples contained from 29 to 98 percent neoplastic cells. In one case, designated OVCA-1, the transfection of high molecular weight DNA extracted from ascites fluid cells induced transformation of NIH/3T3 cells. Since many human carcinomas contain activated *ras*^{K} genes, DNA's

from NIH cells transformed by this ovarian carcinoma DNA were analyzed by the Southern blotting technique and probed for the acquisition of human ras^K DNA sequences. Figure 1 shows that NIH/3T3 cell DNA digested with Eco RI yielded mouse ras^K-containing DNA fragments of 12.0, 6.7, and 1.3 kb (lane a), whereas human DNA (lane b) digested with the same enzyme yielded fragments of 6.6 and 3.0 kb. DNA's from two NIH cell lines transformed by OVCA-1 tumor DNA in primary rounds of transfection (lanes c and d in Fig. 1) and one cell line transformed by NIH transformant DNA in a second round of transfection (lane e in Fig. 1) contained the 3.0- and 6.6-kb human ras^K sequences, in addition to the mouse 12.0-, 6.7-, and 1.3-kb ras^K sequences. Thus, these human ras^K sequences segregated with transforming activity in multiple rounds of transfection, indicating that the transforming gene activated in this ovarian carcinoma was the same ras^K gene previously found in other human carcinomas.

In studies of animal neoplasms, such as bursal lymphomas in chickens (*14*) and mammary carcinomas (*15*) and lymphoid tumors (*11*) in mice, activated transforming genes were detected in tumor cells but not in normal cells of the same animal. In humans, however, this has been documented in only one case. DNA from normal cells of a patient, whose pancreatic carcinoma cells contained an activated ras^K gene, lacked transforming activity in DNA transfection assays (*6*). We could address this problem because the ascites fluid samples from patients with ovarian carcinoma contained normal cells, such as inflammatory and mesothelial cells, in addition to neoplastic cells. When cultured in vitro, the tumor cells did not grow whereas mesothelial cells adhered to culture dishes and divided quite readily (*16, 17*). It has been shown previously (*16*) that mesothelial cells isolated in this manner do not form tumors in nude mice. Additionally, we have distinguished these normal cells from tumor cells by their failure to bind a monoclonal antibody (*18*) that reacts with OVCA-1 tumor cells (data not shown). Therefore, we extracted high molecular weight DNA from cultures of OVCA-1 mesothelial cells and used it in transfection experiments for comparison with DNA from tumor containing ascites fluid from this same patient (Table 1). Unlike DNA from the ascites fluid cells, which transformed NIH/3T3 cells with an efficiency of 0.008 foci per microgram of DNA, DNA from normal mesothelial cells induced transformation at a frequency no greater than the background spontaneous transformation of cultures exposed to control DNA from spontaneously transformed NIH cells (0.002 foci per microgram of DNA). Two foci of transformed NIH cells that appeared after transfection with mesothelial cell DNA (see Table 1) were grown to mass culture for further analysis. Blot-hybridization indicated that these cells lacked human ras^K sequences (data not shown) and thus appeared to be spontaneous transformants. In addition, DNA from NIH/3T3 cells transformed by OVCA-1 tumor DNA displayed a fourfold higher transforming efficiency in secondary rounds of transfection than DNA from the original OVCA-1 cells from ascites fluid (Table 1). The transforming efficiency in secondary transfection assays was similar to those of other activated ras^K genes (*2, 6, 7*). This is consistent with the ascites fluid containing a mixture of tumor cells with activated ras^K and normal cells lacking an activated form of this gene. These results indicate that somatic mutation in the tumor cells, rather than germ line mutation, was responsible for ras^K activation in this human ovarian carcinoma. Since OVCA-1 mesothelial cells expressed *ras* products at normal levels (see Fig. 2), the alternative possibility that the lack of transforming activity of mesothelial cell DNA resulted from hypermethylation of ras^K genes appears unlikely.

Table 1. High molecular weight DNA's were prepared from the ascites fluid of a previously untreated patient (OVCA-1) with serous cystadenocarcinoma of the ovary and from normal mesothelial cells isolated from this same ascites fluid. NIH cells transformed by OVCA-1 DNA [NIH(OVCA-1) cells] were picked and grown to mass cultures for secondary rounds of transfection. Comparable results obtained with two independent transformants were pooled. Each dish of NIH cells was exposed to 20 μg of donor DNA and transformed foci of cells were quantitated 10 to 14 days after transfection (*13*).

Donor DNA	Total number of foci	Total number of recipient cultures	Number of foci per microgram of DNA
OVCA-1 ascites fluid	11	67	0.008
Normal mesothelial cells from OVCA-1	3	72	0.002
Spontaneously transformed NIH cells	2	42	0.002
NIH(OVCA-1) cells	28	42	0.033

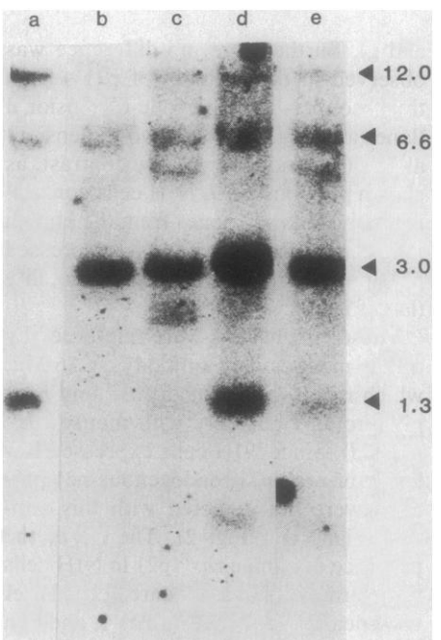

Fig. 1. Ras^K sequences in transformed NIH cells. Cellular DNA's were digested with Eco RI, subjected to electrophoresis in 0.7 percent agarose gels, blotted to gene screen filters, and hybridized to a nick-translated ^{32}P-labeled ras^K probe [1 kb of a *ras* insert of HiHi-3 (*24*), about 4×10^8 count/min per microgram of DNA]. (Lane a) NIH/3T3 cells; (lane b) IMR-90 normal human embryo fibroblasts; (lanes c and d) two independent NIH cell lines transformed by ovarian carcinoma OVCA-1 DNA; (lane e) NIH cells transformed by DNA of NIH(OVCA-1) cells in a second round of DNA transfection. Lengths of ras^K-containing fragments are expressed in kilobases and were calculated from Hind III–digested λ DNA size markers.

Human *ras* genes encode a family of very similar proteins of approximately 21,000 daltons (termed p21's) that bind guanine nucleotides and reside in the plasma membrane (*19*). In the cases studied to date, human *ras* genes acquired transforming activity in tumor cells by genetic lesions that led to structurally altered p21 proteins. For ras^H, two variations have been found. A point mutation in codon 12, substituting a valine for a glycine, occurred in the human bladder carcinoma cell line EJ (*20, 21*), whereas a point mutation in codon 61, changing a glutamine to leucine, was responsible for ras^H activation in the human lung carcinoma cell line Hs242 (*5*). Accordingly, these two cell lines expressed ras^H p21 proteins with abnormal electrophoretic mobilities (*5, 20*).

Activation of ras^K can also occur by

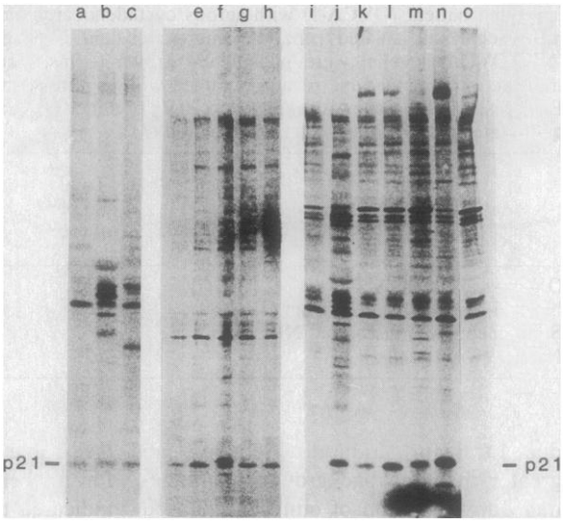

Fig. 2. Expression of human p21 in NIH cells transformed by ovarian carcinoma DNA. Cells were metabolically labeled for 16 to 24 hours with [^{35}S]methionine (> 500 Ci/mmole; 250 μCi/ml, New England Nuclear) in the presence of minimum essential medium containing 10 percent of the normal amount of methionine plus 10 percent calf serum. Portions of cell extracts containing 5×10^6 count/min were immunoprecipitated at 4°C with (lanes a to h) Y13-259 monoclonal antibody to p21; (lanes i to n) YA6-172 monoclonal antibody to p21; or (lane o) normal rat serum as described previously (*7*). (Lane a) Normal mesothelial cells from OVCA-1; (lanes c, h, and i) spontaneously transformed NIH cells; (lanes b and j) HT29 human lung carcinoma cells; (lanes d, k, and o) NIH/3T3 cells transformed by OVCA-1 tumor DNA; (lane g) a second independent line of NIH cells transformed by OVCA-1 DNA; (lanes e and l) NIH cells transformed by NIH(OVCA-1) DNA in a secondary round of transfection; and (lanes m, f, and n) NIH cells transformed by DNA from the human lung carcinoma cell lines Calu-1 (lane m) and LX-1 (lanes f and n).

more than one mutation since two distinct alterations in p21 electrophoretic migration were observed in tumor cell lines containing activated *ras*^{K} (*7*). One abnormal form of p21 was encoded by *ras*^{K} genes activated in the human lung carcinoma cell line LX-1 and in the colon carcinoma cell lines SW480 and SKCO-1. The other form of p21 was encoded by the *ras*^{K} gene activated in the human lung carcinoma cell line Calu-1. Nucleotide sequencing data has confirmed that two different mutations activated *ras*^{K} genes in the SW480 and Calu-1 cell lines. Both involved codon 12, with glycine changed to valine in the former and to cysteine in the latter (*22*).

Since each *ras*^{K} mutation yielded a p21 protein with a unique electrophoretic mobility, we compared the migration in sodium dodecyl sulfate–polyacrylamide gels of p21 encoded by the activated *ras*^{K} in the OVCA-1 cells with that of p21 from other tumor cells containing activated *ras*^{K} genes (Fig. 2). Two monoclonal antibodies to Harvey sarcoma virus *ras*^{H} p21 were used to precipitate p21 from cells metabolically labeled with [^{35}S]methionine. Precipitation with antibody Y13-259 (lanes a to h in Fig. 2), which reacts with all *ras* proteins (*23*), demonstrated that comparable levels of p21's were expressed in normal human mesothelial cells from OVCA-1 (lane a), human tumor cells (HT29) lacking activated *ras*^{K} (lane b), spontaneously transformed NIH cells (lanes c and h), and three independent NIH cell lines transformed by OVCA-1 DNA (lanes d, e, and g). Furthermore, no difference was observed in the mobility of p21's from these sources in either one-dimensional (lanes a to e, g, and h) or two-dimensional gels (data not shown). By contrast, as shown previously (*7*), NIH cells containing activated *ras*^{K} genes from the human lung carcinoma cell line LX-1 expressed p21 proteins with decreased mobility (lane f in Fig. 2).

These conclusions were reinforced by using monoclonal antibody YA6-172, which reacts with human *ras*^{H} and *ras*^{K} p21 proteins but only with mouse *ras*^{H} p21 (*23*). Since NIH cells expressed low levels of *ras*^{H} p21, endogenous p21 proteins were not detected with this antibody (lane i in Fig. 2). Therefore, the presence of human *ras*^{K} p21 in NIH cells transformed by ovarian carcinoma DNA was clearly visualized (lanes k and l in Fig. 2), again at comparable levels and with the same electrophoretic mobility as p21 from human tumor cells lacking activated *ras* genes (lane j). Most important, the mobility of human *ras*^{K} p21 from NIH cells transformed by OVCA-1 DNA differed from the two altered mobility patterns of human *ras*^{K} p21's found previously in NIH cells transformed by other human tumor *ras*^{K} genes. Those two abnormal p21's are represented here in NIH cells transformed by the human lung carcinoma cell lines Calu-1 (lane m) and LX-1 (lane n). Since p21 levels in cells transformed by OVCA-1 DNA were comparable to those in transformed cells lacking activated *ras*^{K}, the most likely mode of *ras*^{K} activation in this tumor was by a mutation that generated a structurally altered p21. Unlike the previously described cases, however, this did not lead to changes in the electrophoretic mobility of p21. Therefore, a previously undescribed mutation appears to be responsible for *ras*^{K} activation in this human ovarian carcinoma. These results further emphasize that a variety of different structural mutations can activate *ras* genes in human tumors. Future experiments will be required to determine how these structural alterations endow p21 with transforming activity.

L. A. FEIG
Dana-Farber Cancer Institute and
Department of Pathology,
Harvard Medical School,
Boston, Massachusetts 02115
R. C. BAST, JR.
Dana-Farber Cancer Institute and
Department of Medicine,
Harvard Medical School
R. C. KNAPP
Brigham and Women's Hospital and
Department of Obstetrics and
Gynecology, Harvard Medical School
G. M. COOPER
Dana-Farber Cancer Institute and
Department of Pathology,
Harvard Medical School

References and Notes

1. G. M. Cooper, *Science* **217**, 801 (1982).
2. C. J. Der, T. G. Krontiris, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3637 (1982).
3. L. F. Parada, C. J. Tabin, C. Shih, R. A. Weinberg, *Nature (London)* **297**, 474 (1982).
4. E. Santos, S. R. Tronick, S. A. Aaronson, S. Pulciani, M. Barbacid, *ibid.* **298**, 343 (1982).
5. Y. Yuasa *et al.*, *ibid.* **303**, 775 (1983).
6. S. Pulciani *et al.*, *ibid.* **300**, 539 (1982).
7. C. J. Der and G. M. Cooper, *Cell* **32**, 201 (1983).
8. M. S. McCoy, J. J. Toole, J. M. Cunningham, E. H. Chang, D. R. Lowy, R. A. Weinberg, *Nature (London)* **302**, 79 (1983).
9. A. Eva, S. R. Tronick, R. A. Gol, J. H. Pierce, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4926 (1983).
10. K. Shimizu, M. Goldfarb, M. Perucho, M. Wigler, *ibid.*, p. 383; K. Shimizu *et al.*, *ibid.*, p. 2112; A. Hall, C. J. Marshall, N. K. Spurr, R. A. Weiss, *Nature (London)* **303**, 396 (1983); M. J. Murray *et al.*, *Cell* **33**, 749 (1983).
11. M.-A. Lane, A. Sainten, G. M. Cooper, *Cell* **28**, 983 (1982).
12. A. Diamond, G. M. Cooper, J. Ritz, M.-A. Lane, *Nature (London)* **305**, 112 (1983); M.-A. Lane, A. Sainten, K. M. Doherty, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
13. T. G. Krontiris and G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1181 (1981).
14. G. M. Cooper and P. E. Neiman, *Nature (London)* **287**, 656 (1980).
15. M.-A. Lane, A. Sainten, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981).
16. Y.-J. Wu *et al.*, *Cell* **31**, 693 (1982).
17. N. Connell and J. G. Rheinwald, *ibid.* **34**, 245 (1983).
18. R. C. Bast *et al.*, *J. Clin. Invest.* **68**, 1331 (1981).
19. R. W. Ellis *et al.*, *Nature (London)* **292**, 506 (1981); M. C. Willingham, I. Pastan, T. Y. Shih, E. M. Scolnick, *Cell* **19**, 1005 (1980); A. Papageorge, D. Lowy, E. M. Scolnick, *J. Virol.* **44**, 509 (1982).
20. C. J. Tabin *et al.*, *Nature (London)* **300**, 143 (1982).
21. E. P. Reddy, R. K. Reynolds, E. Santos, M. Barbacid, *ibid.*, p. 149; E. Taparowsky *et al.*, *ibid.*, p. 762; D. J. Capon, E. Y. Chen, A. O. Levinson, P. H. Seeburg, D. V. Goeddel, *ibid.*

302, 33 (1983); E. P. Reddy, *Science* **220**, 1061 (1983).
22. K. Shimizu *et al.*, *Nature (London)* **304**, 497 (1983); D. J. Capon *et al.*, *ibid.*, p. 507.
23. M. E. Furth, L. J. Davis, B. Fleurdelys, E. M. Scolnick, *J. Virol.* **43**, 294 (1982).
24. N. Tsuchida and S. Uesugi, *ibid.* **38**, 720 (1981).
25. We thank M. Diamond and S. Okenquist for technical assistance, C. Suther for typing the manuscript, and E. Scolnick for providing monoclonal antibodies to p21. Supported by NCI grant CA18689, National Institutes of Health Fellowship CA07101 to L.A.F., an American Cancer Society Faculty Research Award to G.M.C., a Leukemia Society Scholarship to R.C.B., and by funds from the National Cancer Cytology Center and the William P. Graves Ovarian Cancer Research Fund.

24 October 1983; accepted 6 December 1983

Preservation of Membranes in Anhydrobiotic Organisms: The Role of Trehalose

Abstract. *Trehalose is a nonreducing disaccharide of glucose commonly found at high concentrations in anhydrobiotic organisms. In the presence of trehalose, dry dipalmitoyl phosphatidylcholine (DPPC) had a transition temperature similar to that of the fully hydrated lipid, whereas DPPC dried without trehalose had a transition temperature about 30 degrees Kelvin higher. Results obtained with infrared spectroscopy indicate that trehalose and DPPC interact by hydrogen bonding between the OH groups in the carbohydrate and the polar head groups of DPPC. These and previous results show that this hydrogen bonding alters the spacing of the polar head groups and may thereby decrease van der Waals interactions in the hydrocarbon chains of the DPPC. This interaction between trehalose and DPPC is specific to trehalose. Hence this specificity may be an important factor in the ability of this molecule to stabilize dry membranes in anhydrobiotic organisms.*

Trehalose, a nonreducing disaccharide of glucose, is found at particularly high concentrations (as much as 20 percent of dry weight) in many organisms capable of surviving complete dehydration (the so-called anhydrobiotic organisms) (*1–8*). These organisms include spores of certain fungi (*1*), macrocysts of the slime mold *Dictyostelium* (*2*), dry active baker's yeast (*3*), brine shrimp cysts (dry gastrulae of *Artemia salina*) (*4*), and the dry larvae and adults of several species of nematodes (*5*). They can persist in the anhydrobiotic state for many years, but when they come in contact with water they rapidly swell and resume metabolic activities. Survival of dehydration by some of these organisms is correlated with synthesis of trehalose during dehydration (*5, 9*) or its degradation following rehydration (*10*). We report that this molecule alters the physical properties of membrane phospholipids in the dry state in ways that may explain the remarkable stability of membranes in anhydrobiotic organisms.

Dehydration of a biological membrane usually results in such massive upheavals that the structural and functional integrity of the membrane are irreversibly damaged. For example, when vesicles of calcium-transporting membranes are dehydrated, the phospholipids form complex crystalline phases, some of which are nonbilayer structures. Intramembrane particles that represent the calcium-dependent adenosine triphosphatase in these membranes are excluded from these crystals (*11*). Upon rehydration, vesicles show evidence of morphological damage—including fusion between vesicles and redistribution of intramembrane particles—and calcium transport activity is lost (*11*). By contrast, when the membranes are dried in the presence of trehalose at concentrations of at least 20 percent of the dry weight of the membranes (concentrations similar to those found in anhydrobiotic organisms), no evidence of phase transitions is seen during dehydration, and upon rehydration, vesicles are similar morphologically and functionally to freshly prepared ones (*12*). At physiological concentrations, this stabilizing effect is a property of trehalose not shared by other carbohydrates (*13*).

Polar head groups of phospholipids normally are hydrated to some extent, and the head groups are separated from each other by these water molecules (*14*). Thus, when phospholipids are dehydrated, the packing density of the head groups would be expected to increase, thereby increasing opportunities for van der Waals interactions among the hydrocarbon chains. As a result, dehydrated lipids would be expected to enter gel phase at temperatures at which hydrated lipids are in the liquid crystalline phase. Indeed, relative to the hydrated lipid, dry phosphatidylcholine (PC), a phospholipid which is a major constituent of many membranes, has an elevated transition temperature (T_c, the temperature at which the hydrocarbon chains undergo a change from gel to liquid crystalline state) (*15*). Thus, when a membrane is dehydrated at a temperature at which it would normally be in a liquid crystalline state, PC would be expected to enter a gel phase as dehydration progresses. When PC enters a gel phase because of reduced temperature, the result is lateral phase separation of the PC, other lipid classes, and intramembrane

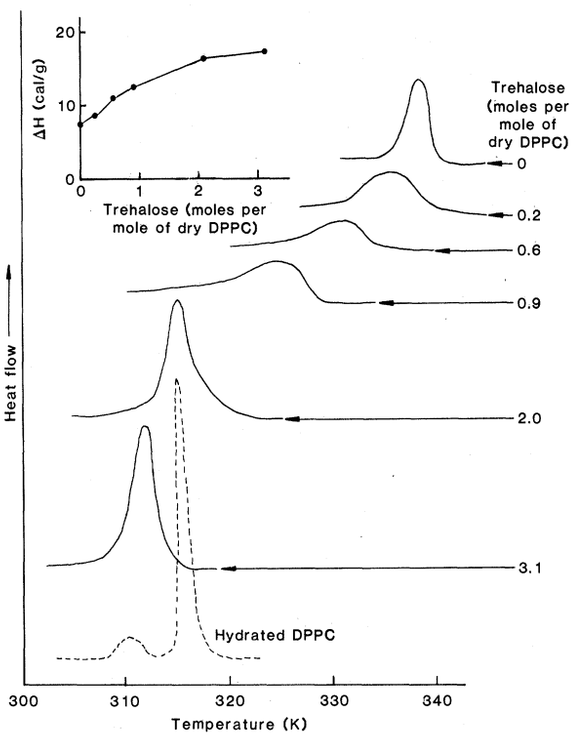

Fig. 1. Differential scanning calorimetry traces of dry and hydrated dipalmitoyl phosphatidylcholine (DPPC) and dry DPPC in the presence of various amounts of trehalose. The inset illustrates the rise in enthalpy of the main phase transition of dry DPPC in the presence of increasing amounts of trehalose (*26, 27*).