nearshore, and offshore zones. Efforts to improve our knowledge of continental shelf ecosystems and of man's impact on them might well receive a boost if we do not underestimate these couplings and explore them in greater detail.

R. E. TURNER

Coastal Ecology Laboratory, Center for Wetland Resources, Louisiana State University, Baton Rouge 70803

S. W. WOO

Skidaway Institute of Oceanography, Skidaway Island, Savannah, Georgia 31406

H. R. JITTS

Division of Fisheries and Oceanography, Commonwealth Scientific and Industrial Research Organization, Post Office Box 21, Cronulla, New South Wales, Australia

References and Notes

1. R. E. Turner, S. W. Woo, H. R. Jitts, *Estuarine Coastal Mar. Sci.*, in press. M. Gilmartin and N. Revelante [*ibid.* **7**, 29, (1978)] have documented a similar strong estuarine influence on coastal phytoplankton communities in the Sea of Cortez during a period of no rainfall or upwelling.
2. J. P. Thomas, thesis, University of Georgia (1966).
3. E. B. Haines, in *Estuarine Research*, L. E. Cronin, Ed. (Academic Press, New York, 1975), p. 303.
4. _____ and W. M. Dunstan, *Estuarine Coastal Mar. Sci.* **3**, 431 (1975).
5. On four cruises, the concentrations of particulate organic carbon on the shelf were 228 mg m^{-3}, and production was 480 mg of carbon per square meter per day (*4*). This is equal to an apparent doubling rate, averaged across the shelf and throughout the 20-m euphotic zone, of 1×10^{-2} doubling per day. This is a very low rate and reflects either high concentrations of detrital material or a low production per unit biomass.
6. The concentration of nitrates often found on the shelf (*3*), for example, are typical of oceanic oligotrophic plankton communities whose cellular physiology is undersaturated with respect to their ability to absorb nitrates [see T. Parsons and M. Takahasi, *Biological Oceanographic Processes* (Pergamon, New York, 1973), p. 90].
7. C. A. Baran and V. G. Burrell, Jr., *S.C. Mar. Res. Center Tech. Rep. 13* (1976), p. 15.
8. Water samples were collected aboard the R.V. *Eastward* on 4 to 30 October 1972, 16 to 22 June 1972, 20 to 28 August 1971, and 9 to 16 November 1971. A submarine photometer was used to select the sampling depths. Productivity measurements were made at in situ temperatures, and two approaches were used. Simulated in situ conditions were created by the use of a gimbaled outdoor chamber fitted with neutral density filters. A fluorescent light bath was used to estimate the maximum rate of photosynthesis (P_{max}). Measurements of apparent net phytoplankton production (particulate only) were obtained by the ^{14}C isotopic dilution method. Inorganic carbon samples were collected for each sample. Duplicate light and dark bottles collected within 60 minutes of local apparent noon (LAN) were inoculated with 1 ml of stock ^{14}C solution at LAN. The incubation period was generally 2 to 3 hours and 0.5 day for fluorescent light and simulated in situ experiments, respectively. After the samples had been filtered through 0.45-μm membrane filters, they were exposed to fuming acid for 3 minutes and their radioactivity was measured with Geiger counters.
9. The linear regression of primary production determined by the simulated in situ method (y, in milligrams of carbon per square meter per 0.5 day) versus the P_{max} (x, in milligrams of carbon per cubic meter per hour) method is given by $y = 0.059 + 0.24\ x$; R^2 (coefficient of determination) = 0.74. The range of y is 0.016 to 0.41.
10. R. E. Turner, unpublished data.
11. Haines (*3*) described a simultaneous increase in chlorophyll and a decrease in salinity as Hurricane Agnes passed through the area in June 1972, which she ascribed (correctly, in our opinion) to the flushing of water from estuaries and the nearshore zone onto the deeper waters of the shelf. On another cruise, similar changes observed after a storm has passed through an area (*4*) were attributed to an increase in the turbulent mixing of nutrients and chlorophyll possibly accumulated at the bottom of the photic zone. L. P. Atkinson (unpublished data) has documented the occasional intrusions of cold, deep Gulf Stream water extending up onto the shelf an average 20 km inside from the shelf break. Nutrients in these intrusions may significantly influence plankton communities by regularly complementing other sources of nutrients and irregularly being mixed with the surface water by storms.
12. K. G. Sellner, R. G. Zingmark, T. G. Miller, *Bot. Mar.* **19**, 119 (1976).
13. D. W. Menzel and J. H. Ryther, *J. Cons. Cons. Int. Explor. Mer.* **26**, 250 (1961).
14. Salinity data for cruises E-22c-71, E-26-71, E-9-72, and E-18b-72 of the R.V. *Eastward* were analyzed. Sections were mapped for the Georgia and South Carolina coast at 5-, 10-, 20-, and 50-fathom contours (1 fathom = 6 feet or ~ 2 m) (*U.S. Geol. Surv. Map 1001*) and from transects perpendicular to the coast and at the St. Mary's, Altamaha, and Savannah rivers and the city of Charleston, S.C. Depth-weighted averages of salinity at each depth contour were estimated and the percent freshwater content was determined by means of a seasonally adjusted standard from water overlying the Gulf Stream.
15. D. S. Gorsline and D. J. P. Swift, in *Continental Shelf Sediment Dynamics: A National Overview* (National Science Foundation, Washington, D.C., 1976), p. 21.
16. D. M. Axelrad, thesis, College of William and Mary (1974); L. R. Pomeroy, L. R. Shenton, R. D. H. Jones, R. J. Reimold, *Limnol. Oceanogr. Spec. Symp.* **1**, 274 (1972); D. R. Heinle and D. A. Flemer, *Mar. Biol.* **35**, 359 (1976); R. E. Turner, *Limnol. Oceanogr.* **23**, 442 (1978).
17. R. E. Turner, *Trans. Am. Fish. Soc.* **106**, 411 (1977).
18. The methods used by each research group varied somewhat. Haines and Dunstan (*4*) incubated their samples longer (24 versus 6 hours), used a rough approximation of light penetration (a secchi disk), and collected samples throughout the day. No single method of measuring primary production is acceptable to all researchers at the present time. These estimates are, at least, a good relative estimate of production across the shelf.
19. W. W. Anderson, J. W. Gehringer, E. Cohen, *U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 178* (1956); *ibid. 198* (1956); *ibid. 210* (1957); *ibid. 234* (1958); *ibid. 248* (1958); *ibid. 265* (1959); *ibid. 278* (1959); *ibid. 303* (1959); *ibid. 313* (1959). Station numbers of the cruises of R.V. *Gill* were grouped as nearshore-shallow (0 to 20 m; stations 23 through 26 and 32 through 44) and deep shelf (20 to 200 m; stations 27 through 31). The same locations were occupied on each of eight cruises. The data shown in Fig. 2 are, therefore, a composite of data from different years combined into 1 year and are a strong argument for the existence of a seasonal pattern.
20. We thank our colleagues at the Skidaway Institute of Oceanography, the Institute of Ecology of the University of Georgia, and the Coastal Ecology Laboratory, Center for Wetland Resources, Louisiana State University. J Gosselink, in particular, was a positive influence during the period of manuscript preparation. This work was supported by the Duke University Oceanographic Program, an Energy Research and Development Administration grant to L. R. Pomeroy, and the Louisiana Sea Grant Program maintained by the National Oceanic and Atmospheric Administrations. The U.S. government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. Coastal Ecology Laboratory, Center for Wetland Resources, Publ. No. LSU-CEL-79-06.

12 February 1979; revised 24 April 1979

Expression of the *Escherichia coli* Cell Division Gene *sep* Cloned in a λCharon Phage

Abstract. *The* Escherichia coli *cell division gene* sep, *which probably codes for one of the penicillin-binding proteins, has been cloned into* λ*Charon 10 to form a viable* sep$^+$ *transducing phage. After infection with this hybrid phage, penicillin-binding protein 3 was overproduced and incorporated into the* E. coli *inner membrane.*

A 20.8-kilobase-pair (kbp) segment of *Escherichia coli* DNA around the minute 2 region of the standard map contains at least seven genes required for cell division, murein biosynthesis, or membrane permeability (*1, 2*). These genes include *sep* and *ftsA*, whose products function during septum formation; conditional mutants defective in these genes grow as long, nonseptate filaments at high temperature. The filaments retain some incomplete constrictions, which represent arrested septation (*2*). The *murE*, *murF*, *murC*, and *ddl* gene products function in murein biosynthesis; temperature-sensitive (ts) mutations in these genes cause lysis at high temperature (*1*). The *envA* mutants have increased permeability to a variety of agents and the cells grow in chains (*3*). All these genes are related in that they participate in cytoplasmic membrane–cell wall synthesis or function. Genetic and physical maps of this group of genes have been prepared by transduction and by heteroduplex analysis of DNA of defective λ transducing phages (Fig. 1A) (*2*).

The *sep* gene probably is identical to the *pbpB* and *ftsI* genes described independently by Spratt (*4*) and Suzuki *et al.* (*5*) and thought to code for penicillin-binding protein 3 (PBP-3), a component of inner membrane (*4*). This conclusion is based on the finding that a *sep* ts mutant lacks PBP-3 activity when assayed in vitro at 30° or 42°C and on a genetic analysis of independently isolated transducing phages (*6*). Therefore, the product of the *sep* (*pbpB*/*ftsI*) gene has two detectable properties—a function during septation and a function in penicillin binding.

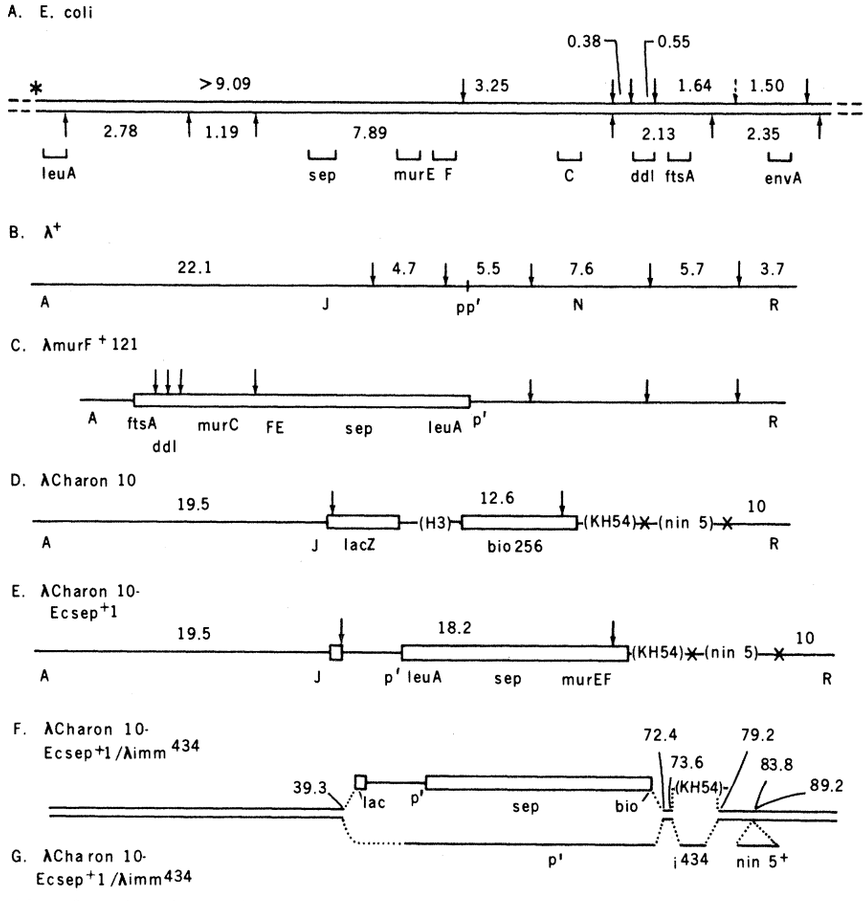
As an approach to defining how septum formation (and consequently cell division) is regulated in *E. coli*, studies on the regulation of expression of *sep* might

0036-8075/79/1012-0220$00.50/0

be useful. The availability of plaque-forming transducing phages that carry bacterial genes can greatly facilitate work on regulation of expression.

We have prepared a viable λ phage carrying the *sep* gene by cloning the *sep* region into λCharon 10. First, a restriction fragment map of the *leuA* to *envA* region was prepared (Fig. 1A). We analyzed *Eco* RI, *Hind* III, and double *Eco* RI and *Hind* III digests of the DNA of defective transducing phages. The mapping was facilitated by the availability of a family of phages with overlapping regions of *E. coli* DNA extending from *leuA* through *envA* (*2*). The *sep* gene is carried on an 18.2-kbp *Eco* RI fragment of λ*murF*$^+$121 DNA (Fig. 1C). The λCharon 10 vector (*7*) (Fig. 1D) was chosen for cloning this fragment for four reasons. (i) It accepts fragments of length 9.2 to 23.4 kbp. (ii) A central fragment must be reinserted to reconstitute infectious molecules. (iii) The presence of the lacZ region permits the identification of phages which have had the λCharon 10 central fragment replaced with foreign DNA. (iv) Although λCharon 10 has a deletion within the *cI* gene, the deletion does not remove any nucleotides required for functioning of O_LP_L or O_RP_R (*8*), which means that λCharon 10 hybrids will transduce efficiently λ$^+$ bacterial lysogens.

The DNA of λ*murF*$^+$121 and DNA of λCharon 10 were cleaved with *Eco* RI, mixed, ligated with T4 DNA ligase, and used to transfect $CaCl_2$-treated *E. coli* C600 cells. The transfected cells were plated on medium containing 5-chloro-4-bromo-3-indolyl-β-D-galactoside (XG) to identify the phages in which the lacZ region was replaced by DNA from λ*murF*$^+$121 and, consequently, grew as colorless plaques. λCharon 10 plaques



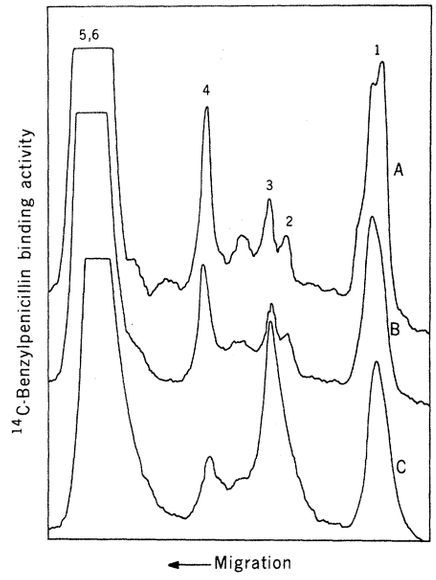


Fig. 1 (left). Genetic and physical maps of *E. coli* and λ phages. *Eco* RI and *Hind* III sites are represented by ↓ and ↑, respectively. Phage DNA is represented by one solid line; *E. coli* DNA by open bars. (A) Restriction fragment map of the *leuA-envA* region of *E. coli*. Sizes of the fragments are given in megadaltons. The fragment map was prepared by analysis of cuts made by *Eco* RI, *Hind* III (both from Bethesda Research), and a mixture of *Eco* RI and *Hind* III in the DNA of defective transducing phages λ*leuA*$^+$13, λ*sep*$^+$82, λ*sep*$^+$27, λ*sep*$^+$3, λ*ddl*$^+$24, λ*murF*$^+$121, and λ*sep*$^+$46 (*2*). The asterisk (*) indicates the position of prophage integration in strain 73, a secondary site lysogen from which defective transducing phages were prepared (*2*). The broken arrow indicates that the order of the 1.64- and 1.5-megadalton *Eco*RI fragments has not yet been proved. The approximate gene positions are indicated, based on analysis of heteroduplexes of defective λ*sep*$^+$ phages (*2*) and on the identity of *Eco* RI and *Hind* III fragments cut from each phage DNA. (B) Map of λ DNA including *Eco* RI sites. Fragment sizes are given in kilobase pairs (*9*). (C) Map of λ*murF*$^+$121 DNA including *Eco* RI sites. The *sep*-containing fragment was 18.2 kbp. (D) Map of λCharon 10 from (*7*). The numbers are fragment sizes in kilobase pairs. Deletions are represented by parentheses. H3 extends from 52.6 to 57.2 percent (*7*), KH54 from 74.1 to 78.4 percent (*8*), and *nin*5 from 83.8 to 89.2 percent of λ (*10*). Altered *Eco* RI sites are represented by X. (E) Map of λCharon 10-Ec*sep*$^+$1. This hybrid DNA was constructed by the procedures of Cameron *et al.* (*11*), except that T4 DNA ligase (Miles) was used. Portions of λ*murF*$^+$121 or λCharon 10 DNA (4 μg in 50 μl) were cut by treatment with four units of *Eco* RI (two additions; 20 minutes at 37°C). After being heated, 2 μg of cut λCharon 10 DNA and 4 μg of cut λ*murF*$^+$121 DNA were mixed in 100 μl and incubated with four units of T4 DNA ligase (in the presence of 60 μ*M* ATP) at 10°C for 18 hours. The mixture was used to transfect strain C600 (*12*). Transfected cells were plated on NZ amine agar containing XG (*7*). (F) Diagram of heteroduplexes of DNA of λCharon 10-Ec*sep*$^+$1 and λ*imm*^{434}. Two close parallel single lines represent duplex λ DNA, separated lines are single strands, and the bars are *E. coli* DNA. Dots show continuity. Numbers represent the percentage of λ DNA from the left end (*7, 10*). (G) Electron micrograph of a heteroduplex as diagrammed in F. Fig. 2 (right). Overproduction of penicillin-binding protein 3 after infection by λCharon 10-Ec*sep*$^+$1. Inner membrane proteins of 3-liter cultures of strain 2e01c growing at 30°C which were uninfected (A), infected with λ*cI*857*S*am7 (multiplicity of infection of 5) (B), or infected with λCharon 10-Ec*sep*$^+$1*S*am7 (multiplicity of infection of 2) (C) were isolated and assayed for binding of [^{14}C]benzylpenicillin binding activity (*4*). The PBP's were separated by electrophoresis on a 7.5 percent polyacrylamide gel which was dried and exposed to sensitized x-ray film for 1 month. The binding activity was measured by scanning with a Joyce-Loebl densitometer and integrating the areas under the peaks with a Hewlett-Packard digitizer and calculator. The PBP's are numbered according to Spratt (*4*).

are blue on XG plates because β-galactosidase is present (*7*).

Colorless plaques were picked and tested for ability to transduce a *sep* ts(λ$^+$) lysogenic recipient to temperature insensitivity (Ts$^+$) (*2*). One viable *sep*$^+$ transducing phage, designated λCharon 10-Ec*sep*$^+$1, was further characterized. This hybrid phage (Fig. 1E) was also able to transduce *leuA*, *murE*, and *murF* mutant recipients, and thus carries these three genes as well as the *sep* gene. λCharon 10-Ec*sep*$^+$1 DNA was cleaved by *Eco*RI into three fragments—19.5, 18.2, and 10 kbp in length; and its structure was shown by heteroduplexing the DNA with λ*imm*^{434} DNA (Fig. 1G). A diagram of the λCharon 10-Ec*sep*$^+$1/λ*imm*^{434} molecules is presented in Fig. 1F.

Although the *sep* fragment could have been inserted in either of two orientations (that is, λ*J*, λP′, *leuA sep murE F* λ*R*, or λ*J murF E sep leu* λP′ λ*R*), the actual orientation was the one diagrammed: λ*J* λP′ *leuA sep murE F* λ*R*. This conclusion is based on analysis of two types of heteroduplexes. First, the λCharon 10-Ec*sep*$^+$1/λ*imm*^{434} heteroduplex molecules contained no duplex DNA in the P′ region; if the orientation were λ*J murF E sep leuA* λP′ λ*R*, reannealing would have occurred between the P′ regions of both single strands. Second, heteroduplexes of λCharon 10-Ec*sep*$^+$1 and λ*sep*$^+$82 DNA's were prepared. λ*sep*$^+$82 carries a 10.2-kbp insertion of *E. coli* DNA in which *sep* and *leuA* are in the same orientation as in λ*murF*$^+$121 (Fig. 1C). Heteroduplexes of λ*sep*$^+$82/λCharon 10-Ec*sep*$^+$1 DNA contained a central single-stranded loop in which there was no homology between the *sep-leuA* DNA of λ*sep*$^+$82 and the *leuA-sep* region of λCharon 10-Ec*sep*$^+$1. Therefore, the hybrid phage (λCharon 10-Ec*sep*$^+$1) contains the 18.2-kbp fragment in the orientation which is opposite to that in λ*sep*$^+$82 or λ*murF*$^+$121 (Fig. 1C).

After infection of wild-type *E. coli* by the hybrid phage, penicillin-binding protein 3 was overproduced (Fig. 2). Cells were grown at 30°C and infected with a lysis-defective derivative (*S*am7) of λCharon 10-Ec*sep*$^+$1. Control cells were infected with λ*cI*857*S*am7. Two hours after infection, membrane fractions were isolated and [^{14}C]benzylpenicillin was bound to the membrane proteins (*4*). After treatment with Sarkosyl, the inner membrane proteins were separated by electrophoresis on a polyacrylamide slab gel, and the individual PBP's were assayed by fluorography. Membranes of uninfected cells and cells infected with λ*cI*857 contained normal amounts of the six PBP's, but in cells infected with the hybrid λCharon 10-Ec*sep*$^+$1, PBP-3 was overproduced. The level of PBP-3 in membranes of the hybrid-infected cells was approximately four to five times as high as that in membranes of λ*cI*857-infected or -uninfected cells. Therefore, PBP-3 was overproduced and excess PBP-3 was able to incorporate into inner membrane. The extent of overproduction (that is, total soluble and membrane-bound PBP-3) could not be determined because of the insensitivity of the assay; therefore, the four- to fivefold overproduction is a minimum estimate.

In addition to allowing the amplification of the *sep* gene for the production of PBP-3 for chemical studies of the protein, use of the hybrid phage should facilitate genetic studies of the *sep* region because mutants of *sep* can readily be isolated from λCharon 10-Ec*sep*$^+$1. Although *sep* is an essential gene from *E. coli*, it is not required for growth of λ. Both nonsense and deletion mutations can be expected in the *sep* region. In addition, the hybrid phage should be useful in studying the mechanism of insertion of PBP-3 into the inner membrane. DNA of the hybrid phage could also be used as a probe to measure *sep* messenger RNA synthesis in a study of regulation of *sep* expression.

CARLEEN A. IRWIN, GAIL FLETCHER
CYNTHIA L. SILLS, JAMES R. WALKER
Department of Microbiology,
University of Texas, Austin 78712

References and Notes

1. H. J. W. Wijsman, *Genet. Res.* **20**, 65 (1972); *Mol. Gen. Genet.* **147**, 99 (1976).
2. G. Fletcher, C. A. Irwin, J. M. Henson, C. Fillingim, M. M. Malone, J. R. Walker, *J. Bacteriol.* **133**, 91 (1978).
3. S. Normark, *Genet. Res.* **16**, 63 (1970).
4. B. Spratt, *J. Bacteriol.* **131**, 293 (1977).
5. H. Suzuki, Y. Nishimura, Y. Hirota, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 664 (1978).
6. M. Iwaya, D. J. Tipper, F. P. Câmara, C. Madureira, D. F. de Almeida, C. L. Sills, J. R. Walker, in preparation.
7. F. R. Blattner, B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. -A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, O. Smithies, *Science* **196**, 161 (1977).
8. F. R. Blattner, M. Fiandt, K. K. Hass, P. A. Twose, W. Szybalski, *Virology* **62**, 458 (1974).
9. P. Philippsen, R. A. Kramer, R. W. Davis, *J. Mol. Biol.* **123**, 371 (1978).
10. M. Fiandt, Z. Hradecna, H. A. Lozeron, W. Szybalski, in *The Bacteriophage Lambda* (Cold Spring Laboratory, Cold Spring Harbor, N.Y., 1971), pp. 329-354.
11. J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
12. M. Mandel and A. Higa, *J. Mol. Biol.* **53**, 159 (1970).
13. Supported by NSF grant PCM 78-07808, NIH grant AI08286, and in part by American Cancer Society grant NP169B. C.A.I. is an NSF predoctoral fellow.

26 March 1979; revised 4 June 1979

Calcification of Differentiating Skeletal Mesenchyme in vitro

Abstract. *Embryonic limb-bud mesenchyme was induced to calcify in culture by the addition of 3 m*M *inorganic phosphate to the medium. Phosphate enhanced calcification of the matrix produced by mesenchymal or fibroblast-like cells, whereas no calcification was evident in areas where cartilage had developed. However, calcification was induced throughout the cell layer by altering the cartilage matrix properties with certain enzymes or by changing the phenotypic expression of the cells with vitamin A.*

The factors that initiate and control the deposition of minerals in animal tissue are not well understood. In general, the production of a calcifiable matrix is required prior to mineral deposition (*1*). In bone, type I collagen is laid down and mineral is then deposited within it (*1*). In cartilage, deposition of mineral is associated with regions of the cartilage from which proteoglycans have been removed (*2*). It has been shown that rat embryonic bone cells (*3*) and embryonic chick limb-bud cells (*4*) are capable of depositing a matrix that calcifies in vitro. However, cellular control of the amount or distribution of calcified matrix in these culture systems has not been demonstrated.

The system we have developed allows control of both the amount and distribution of mineralization. Embryonic chick limb-bud mesenchymal cells were prepared in micro-mass cultures by placing a 20-μl drop containing 4 × 10^5 cells onto a 35-mm tissue culture dish and allowing the cells to attach themselves before adding the medium [Eagle's minimum essential medium (MEM) supplemented with 10 percent fetal calf serum] (*5*). The mesenchymal cells undergo chondrogenesis (formation of cartilage) within 4 days. Chondrogenesis is characterized by the accumulation of an extracellular matrix consisting primarily of type II [α1 $(II)_3$] collagen (*6*) and proteoglycan aggregates (*7*). The appearance and organization of these matrix components in the limb mesenchyme cultures has been well documented (*8*). The 4-day-old cultures contain a central zone of cartilage nodules that coincide with the region of initial mesenchymal cell attachment and an outer halo of spindle-shaped cells (mes-

0036-8075/79/1012-0222$00.50/0