dependent. Cystine egress is not influenced by 1 m*M* NEM and proceeds without intermediary conversion to cysteine (Table 1). Since methionine, tryptophan, and leucine egress are not substantially altered in cystinotic lysosomes (*8, 9*), these amino acids probably do not share a lysosomal transport mechanism with cystine.

The demonstration of a natural cystine transport system in lysosomes, and the availability of mutant lysosomes wholly and partially deficient in such transport, should permit elucidation of fundamental information on this aspect of lysosomal function. Furthermore, the possibility is suggested that other lysosomal transport systems may exist and that their dysfunction could play a role in certain other lysosomal storage diseases.

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Transmethylation of Phosphatidylethanolamine: An Initial Event in Embryonic Chicken Lens Fiber Cell Differentiation

Abstract. *Agents that induce differentiation of lens epithelial cells into lens fiber cells in vitro transiently stimulate the transmethylation of phosphatidylethanolamine. Inhibition of transmethylation by 3-deazaadenosine results in a corresponding inhibition of the cell elongation that characterizes lens fiber formation, suggesting that phospholipid methylation plays an essential role in the differentiation of these cells.*

Explants of lens epithelium from chicken embryos in early development differentiate to form lens fibers when cultured in the presence of fetal calf serum (*1, 2*), vitreous humor (*3*), or insulin (*4*). As the cells differentiate in vitro they undergo the morphological, ultrastructural, and biochemical changes that characterize lens fiber cell formation in vivo (*5, 6*). The cells elongate (*1–4*), cease dividing (*7, 8*), and accumulate messenger RNA for the lens-specific protein δ-crystallin (*9, 10*), thus becoming highly specialized for δ-crystallin synthesis (*2, 10, 11*). Although the initial events leading to lens fiber cell formation are not known, it is likely that a cell surface receptor is involved, since both insulin and a 60,000-dalton protein present in the vitreous humor (*3*) are capable of producing lens fiber formation in vitro. Recently it was shown that a transient stimulation of the transmethylation of phosphatidylethanolamine to phosphatidylcholine is an early event associated with the binding of a number of proteins and peptides to their respective cell surface receptors (*12*). Phospholipid methylation is thought to initiate a chain of events leading to a cellular response, such as secretion in mast cells, chemotaxis in leukocytes, and neuritic outgrowth in cultured superior cervical ganglia (*12*). The present study was undertaken to determine whether the transmethylation of phosphatidylethanolamine to phosphatidylcholine is involved in the initiation of lens fiber cell differentiation.

Square explants 0.7 mm on a side and containing approximately 10^4 cells were cut from the central region of the lens epithelium of 6-day-old embryonic chickens (*2*). A differential interference contrast microscope was used to measure cell length in living explants 1 and 2.5 hours after stimulation (*13*). For each

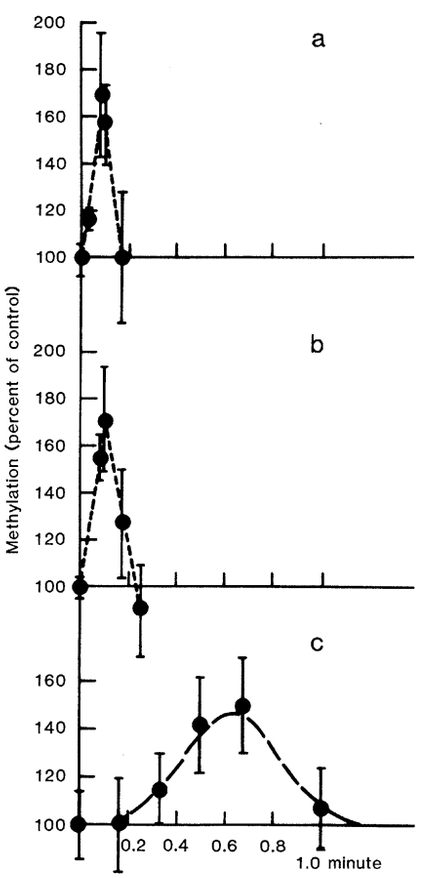

Fig. 1. Incorporation of ^3H from L-[methyl-^3H]methionine into phosphatidylcholine as a function of time after the addition of (a) fetal calf serum, (b) embryonic chicken vitreous humor, or (c) bovine insulin. Each data point is the mean ± standard error for 6 to 30 measurements.

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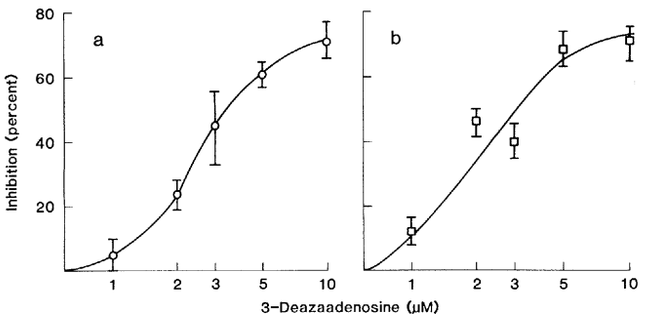

Fig. 2. Inhibition of (a) phospholipid methylation and (b) cell elongation by 3-deazaadenosine. Phospholipid methylation data are means ± standard errors for four measurements. Cell elongation data, calculated from measurements made 1 hour after a 1-minute exposure of the lens epithelial explants to embryonic chicken vitreous humor, are means ± standard errors for 20 measurements.

measurement of phospholipid methylation, six to ten explants were cultured for 1 hour at 37°C in 1 ml of Ham's F-10 medium (*14*) containing 0.1 mCi of L-[methyl-^{3}H]methionine (specific activity, 78 Ci/mmole). The atmosphere was 95 percent air and 5 percent CO_2. At the end of the hour the explants were stimulated to differentiate by adding an appropriate volume of fetal calf serum (final concentration, 15 percent), bovine insulin (final concentration, 1 μg/ml), or chicken vitreous humor (*3*) (final concentration, 50 percent). Control dishes received equivalent amounts of Ham's F-10 medium. At specified times the tissues were fixed by adding 50 percent trichloroacetic acid (150 μl/ml). Phospholipids were extracted from the tissues with $CHCl_3$ and CH_3OH (1:1) (*15*) or *n*-butanol (*16*), and the extracts were chromatographed in two dimensions on silica-gel H thin-layer plates by using the solvent system of Anderson *et al.* (*17*). Phosphatidylcholine and lysophosphatidylcholine were scraped from the plates and radioactivity was determined by scintillation counting. Since the transfer of radioactivity from [^{3}H]methionine to phosphatidylcholine occurs by the stepwise methylation of phosphatidylethanolamine with *S*-adenosylmethionine as the methyl donor (*18*), the radioactivity in phosphatidylcholine provides a measure of the activity of the methylation pathway.

Incorporation of radioactivity into phosphatidylcholine in the cultured explants increased seconds after the addition of fetal calf serum or embryonic chicken vitreous humor (Fig. 1, a and b). Phosphatidylcholine labeling reached a peak after 6 seconds and returned to the baseline value after 15 seconds. Insulin also transiently stimulated the accumulation of ^{3}H-labeled phosphatidylcholine but with a slower time course, reaching a maximum after about 30 seconds (Fig. 1c). Control cultures showed no increase in phosphatidylcholine labeling. Low levels of radioactivity were consistently found in lysophosphatidylcholine, indicating that the phosphatidylcholine formed by the methylation pathway was subsequently degraded by phospholipase A (*19*).

To determine whether phospholipid methylation is related to the initiation of differentiation in these cells, we tested the ability of the methylation inhibitor 3-deazaadenosine (*20*) to inhibit the cell elongation that characterizes lens fiber formation. 3-Deazaadenosine (1 to 10 μ*M*) inhibited phospholipid methylation (Fig. 2a) and also inhibited the cell elongation produced by a 1-minute exposure to embryonic chicken vitreous humor. At each concentration of 3-deazaadenosine, the inhibition of elongation closely corresponded to the inhibition of phospholipid methylation (Fig. 2b), suggesting that the drug inhibits both processes by acting at a single site (*21*). The median inhibitory concentration was approximately 3 μ*M* for both phospholipid methylation and cell elongation. Identical results were obtained when vitreous humor was replaced by fetal calf serum or insulin. Complete blockage of phospholipid methylation by a combination of 10 μ*M* 3-deazaadenosine and 100 μ*M* homocysteine thiolactone (*20*) totally inhibited cell elongation. The inhibition was reversible, with the cells recovering their ability to elongate in response to chicken vitreous humor within 3 hours after removal of the drugs.

In view of the close correspondence between the dose response curves for inhibition of phospholipid methylation and lens cell elongation, it seems likely that 3-deazaadenosine affects cell elongation by inhibiting phospholipid methyltransferases. 3-Deazaadenosine, like other structural analogs of *S*-adenosylhomocysteine, causes an intracellular accumulation of *S*-adenosylhomocysteine, which inhibits all methyltransferases that utilize *S*-adenosylmethionine as a methyl donor; however, different methyltransferases vary greatly in their susceptibility to inhibition (*20*). Phospholipid methylation is especially sensitive to inhibition by 3-deazaadenosine in other cell types, with median inhibitory concentrations in the 1 to 10 μ*M* range (*22*). In contrast, comparable inhibition of protein carboxymethylation and nucleotide methylation requires concentrations of the drug at least an order of magnitude greater (*22*). Furthermore, it is improbable that nucleic acid methylation plays a regulatory role in lens fiber formation, since significant cell elongation can occur in the absence of RNA and protein synthesis (*23*). The present results thus suggest that a transient increase in phospholipid methylation is a key step in the differentiation of embryonic chicken lens epithelial cells into lens fibers. Since regulation of the phospholipid methylation pathway occurs seconds after the stimulation of lens fiber formation in vitro, it is possible that this biochemical event initiates the subsequent differentiation of the cells.

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Scaling in Tensile "Skeletons": Structures with Scale-Independent Length Dimensions

Abstract. *Skeletal structures that resist only tensile forces can scale differently than compression resisting structures that fail in bending or buckling. The tensile structures examined scalelike simple ropes: length and diameter of the structure are not correlated, and in three of four cases, length is independent of scale or load, but diameter is dependent on scale. These relations suggest that similarity in stress rather than strain, or deformational behavior, is the basis for mechanical adaptation in the gross dimensions of these tensile structures.*

Skeletons resist forces in the environment as well as forces generated by the organism. Some skeletal tissues, such as wood and bone, are rigid and resist both tension and compression; others, such as tendons and some plant stalks, are fibrous, flexible, and resist only tensile loads. The support system of most higher organisms combines tensile, compression resistant, and pliant elements (*1*). The combination of these elements in a particular skeleton reflects the size, behavior, and evolutionary history of the organism. One aspect of the size component in skeletal design that can be somewhat isolated from the other components is scaling—the adaptation of skeletal dimensions to the magnitude of environmental forces or the mass of the organism (*2, 3*). Modes of mechanical failure underlie most models and empirical studies of scaling, and scaling criteria have been based on similarity in stress (that is, force per unit of cross-sectional area of the skeletal element is constant throughout the size range, so that the diameter of the element is proportional to the load) or in strain (that is, deformation in length and diameter remains proportional to load; this might be termed a "work-to-break" criterion) (*2, 4*). Models for rigid skeletons based on either stress or strain criteria require similar scaling relationships, only the predicted exponents differ. Rigid, compression resisting skeletons scale so that (i) the length (y) and the diameter (x) of the elements are correlated and related by the allometric equation $y = ax^b$, where a is a constant and b has a value between 0.5 and 1.0, and (ii) both length and diameter scale with load or body mass (*2, 4, 5*).

The scaling of tensile skeletons can be significantly different from that of bones or other rigid elements. Galileo noted that the length of a tensile structure, such as a rope or cable, may be independent of diameter and load (*6*). Stress in a tensile element is a function only of its diameter and the load. If stress is the criterion of similarity, (i) biological tensile "skeletons" should exhibit variation in length which is independent of diameter, and (ii) diameter should scale with load, but length should be independent of load. In contrast, if strain energy or the deformation under load is the criterion of similarity, then (i) variation in length and diameter should be correlated, and (ii) both dimensions of the element should scale with the load.

We examined the scaling of length and diameter in four tension resisting structures (Fig. 1). In all but one case the relationships of the skeletal dimensions and estimates of the tensile loading were also examined. Whether there are scaling relationships among length, diameter, and load can be evaluated from the correlation coefficient. The scaling relationships are described by the least squares fit of the data, in logarithmic form, to the allometric equation. The

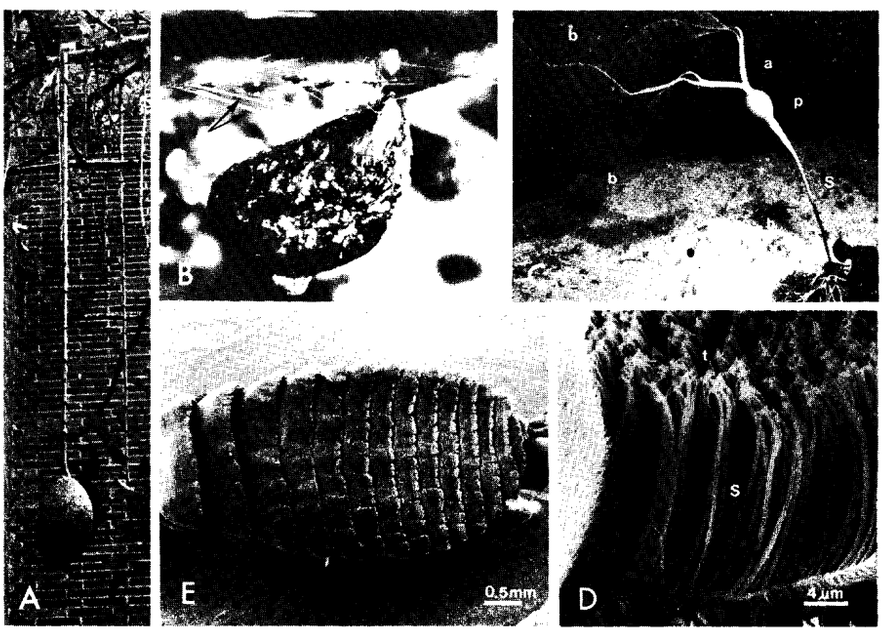

Fig. 1. (A) The stalk and fruit of the sausage tree, *Kigelia pinnata*; the arrow indicates the attachment of the stalk to the tree branch. (B) The blue mussel, *Mytilus edulis*, suspended from a glass plate by byssus threads (black arrows) that are independently cemented to the substrate and arranged like guy wires. (C) The elk kelp, *Pelagophycus porra*. The root-like holdfast (*h*) anchors the plant to the sea floor; the blades (*b*), which can be as long as 12 m, stream out from the "antlers" (*a*); the antlers join at the gas-filled pneumatocyst (*p*); and between the "float" and the holdfast is the long, extensible stipe (*s*). (D) The subdigital hairs or setae of *A. evermanni*. Body weight is suspended from the spatulate setae tips (*t*) by the long stalks (*s*); the mechanism involved in grip is not known (*13*). This is a ventrolateral view of the setae taken near the distal edge of a lamella with the claw toward the right; note the variation in stalk length. (E) The adhesive toe pad of *Anolis cristatellus*, consisting of modified scales or lamellae (*l*); the claw (not shown) is toward the right.

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