If triggering of *Mu* is chronological and occurs before pollen differentiation, the total mutation frequency should also be higher in later pollen samples, inasmuch as *Mu* would have had a longer time to act between triggering and pollen differentiation. Such higher rates are not observed consistently in later shedding pollen. However, a chronological trigger, combined with different maturity rates, might account for the significant difference in total mutant frequency observed among the five crosses (Table 1). In a slow-maturing plant, the chronological trigger would result in an earlier triggering of *Mu* activity, relative to the development of the tassel, than in a faster maturing plant. A slow-maturing plant thus could have more mutants than a faster maturing plant. As indicated in Tables 1 and 2 the five plants were drawn from two different families, designated 5061 and 5062. Family 5061 was planted a week earlier than 5062. As is usually the case with delayed planting, the later plants matured a little faster than the earlier ones. Family 5062 began shedding pollen 4 to 5 days after 5061. Since the 5061 plants matured a little more slowly, they would be expected to have a higher mutation frequency if a chronological trigger were involved. The mutation frequency for the total of the 5061 crosses was 12.9 percent and for the total of the 5062 crosses was 15.4 percent. The faster maturing plants had the higher frequency, although a chi-square test indicated that the two populations are not significantly different. The slower maturing plants also would be expected to have more clusters, and thus an association between higher mutation rate and the occurrence of clusters would be expected. No consistent relation between these two phenomena is seen (Table 1). Thus, the rate of maturation is not a likely explanation for the different mutation frequencies. The reason for the different rates probably will not be known until more is learned about the nature of the *Mu* system.

In sum, these results indicate that a developmentally timed trigger for *Mu* activity is more likely than a chronologically timed trigger.

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References and Notes

1. D. S. Robertson, *Mutat. Res.* **51**, 21 (1978).
2. ———, *Genetics* **94**, 969 (1980).
3. T. A. Kiesselbach, *Nebr. Agric. Exp. Stn. Res. Bull. No. 161* (1949).
4. O. Kempthorne, *An Introduction to Genetic Statistics* (Iowa State Univ. Press, Ames, 1969), pp. 153–154.

$$\chi^2 = \frac{\sum_i (p_i n_{i1} - p N_{\cdot 1})}{\bar{p}\bar{q}}$$

where p_i is the frequency of mutations in each day's outcross, n_{i1} is the total number of mutant plants from each day's outcross, $\bar{p}$ is the frequency of mutations in the total population tested for heterogeneity, $N_{\cdot 1}$ is the total number of plants with mutants in the whole population tested, and $\bar{q} = 1 - \bar{p}$.
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Adenosine Triphosphate–Deficient Erythrocytes of the Egg-Laying Mammal, Echidna (*Tachyglossus aculeatus*)

Abstract. *The erythrocytes of the short-beaked echidna* (Tachyglossus aculeatus), *an egg-laying mammal, were examined for the presence of phosphorylated compounds. The erythrocytes contained only 0.03 ± 0.01 micromoles of adenosine 5′-triphosphate per milliliter of cells. This amount is two orders of magnitude less than that in human cells. Although the echidna erythrocytes had an abundance of 2,3-diphosphoglycerate and other glycolytic intermediates, no other energy-rich pyridine and purine compounds were detected.*

Metabolic machinery in mature mammalian erythrocytes is limited in that the regeneration of adenosine 5′-triphosphate (ATP) depends solely on glycolytic pathways. Although bloodborne glucose is believed to be the key metabolic substrate for mammalian erythrocytes, there are exceptions; for example, in the pig, erythrocytes appear to depend for survival mainly on inosine derived from the liver and other organs (*1–3*). Functions in which ATP plays an essential role include maintaining cell shape (*4, 5*), regulating cell volume by means of active cation transport (*6*), decreasing oxygen affinity of hemoglobin (*7, 8*), and phosphorylating metabolic substrates (*9*). Among vertebrates, the ATP concentration in erythrocytes is highest in the snake (15 μmole per milliliter of cells) (*10*) and lowest in the horse (0.2 μmole/ml) (*11*). About 15 years ago, a curious phenomenon indicating the almost complete absence of ATP in erythrocytes of the short-beaked echidna, an egg-laying mammal, was noted (*12*), but no data were reported. Attempts to corroborate this observation proved to be difficult, owing to the scarcity of echidnas in the United States.

We have now determined the distribution of phosphorylated compounds in erythrocytes of the echidna. Ten echidnas weighing 1.5 to 3.0 kg were studied; some of the animals were collected near Hobart, Tasmania, with permission of the office of the National Parks and Wildlife Services, and others were brought to the University of Tasmania by local residents. The animals were lightly anesthetized with halothane. A blood sample, which varied from 5 to 10 ml, depending on body weight, was taken by cardiac puncture, with EDTA used as an anticoagulant. All animals survived this procedure and remained healthy. A portion of the whole blood sample was taken for measurement of hematological indices, and the rest was used for studies of membrane transport and metabolism (*13*). For the determination of intermediary metabolites, blood samples from four animals were used. The erythrocytes were washed in isotonic NaCl several times, with the usual care taken to aspirate the buffy coat. At least 0.2 ml of freshly packed cells were extracted with 0.56*M* perchloric acid and neutralized with K_2CO_3. The neutralized extract was immediately frozen, packed in dry ice, and transported by air carrier to the University of Alabama. The extract remained frozen until it was assayed for metabolites.

Glycolytic intermediates and cofactors in the extract were determined by Bessman's procedure for anion-exchange column chromatography (*14, 15*). A 3 by 150 mm column partially filled with Dowex AG1-X4 resin and a 3 by 500 mm column packed with equal portions of DA-X4-20 (Durrum) and Aminex A-25 (Bio-Rad) resins were used in tandem. A tenfold dilution of the extract was placed on the columns and eluted with 160 ml of a linear gradient of ammonium chloride (0.1*M* to 0.6*M*) containing 0.5*M* potassium tetraborate. The eluant was monitored at 254 nm with a spectrophotometer (ISCO). Eluant fractions (75 μl) were collected in 48 cups, ashed, and assayed colorimetrically for inorganic phosphate. The temperature of the ashing chamber was gradually increased from 22° to 400°C for complete ashing of organic phosphates. The resulting inorganic phosphate was assayed colorimetrically by addition of 0.5 ml of a solution composed of 20 percent ascorbic acid and 2.5 percent ammonium molybdate in 2.5 percent sulfuric acid to each cup. A fully developed color mixture was monitored

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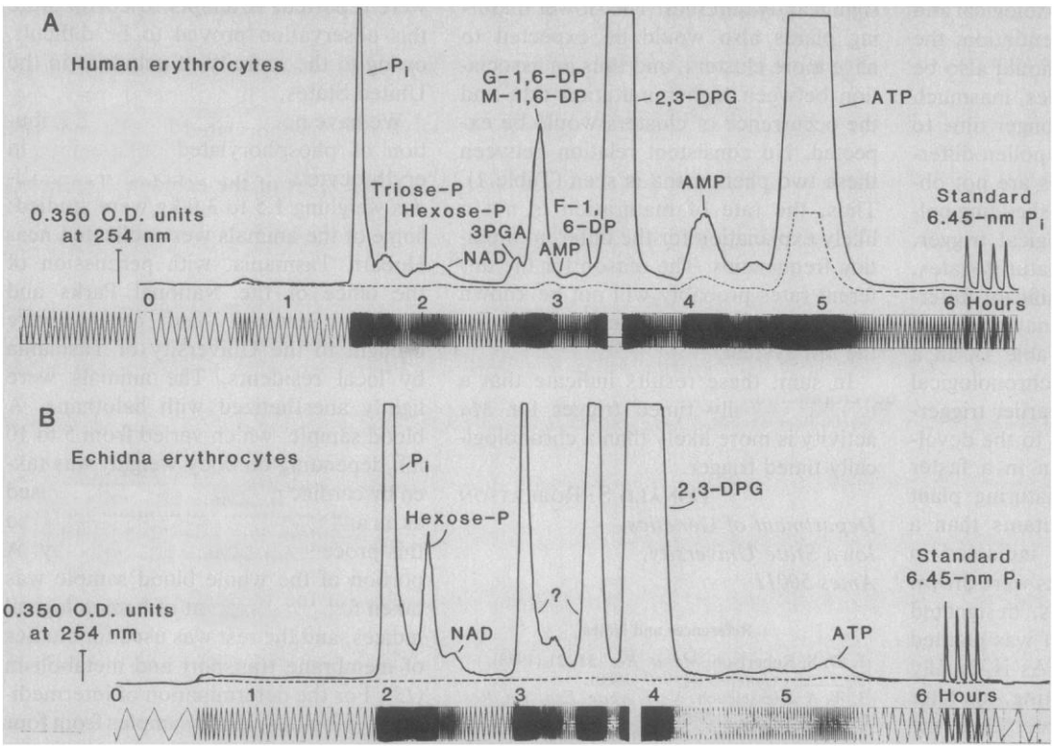

Fig. 1. Phosphorylated compounds in human and echidna erythrocytes. (A) Human cells (0.225 ml) and (B) echidna cells (0.220 ml) were extracted and analyzed by anion-exchange column chromatography. (——) Phosphate recording; (- - - -) ultraviolet recording. For details, see text. Abbreviations: *O.D.*, optical density; *Triose-P*, triose phosphate; *Hexose-P*, hexose phosphate; P_i, inorganic phosphate; *NAD*, nicotinamide adenine dinucleotide; *G-1,6-DP*, glucose 1,6-diphosphate; *M-1, 6-DP*, mannose 1,6-diphosphate; *F-1,6-DP*, fructose 1,6-diphosphate; *AMP*, adenosine 5′-monophosphate; *ADP*, adenosine 5′-diphosphate; *ATP*, adenosine 5′-triphosphate; *2,3-DPG*, 2,3-diphosphoglycerate; and *3PGA*, 3-phosphoglycerate.

at 830 nm on a spectrophotometer (Glenco) coupled with a recorder (Linear Instruments). Phosphorylated compounds were identified by their elution positions and quantitated by integration of the area of each phosphate peak. A firefly enzyme assay for ATP and a chromotropic acid assay (*16*) for diphosphoglycerate were also carried out on the perchloric acid extracts.

Figure 1 shows a typical ion-exchange column chromatogram of phosphorylated compounds in human erythrocytes and in erythrocytes obtained from four short-beaked echidnas. The glycolytic intermediates of human cells are well known (*14, 15*) and are included for comparison. One of the most striking features of the echidna cells is the virtual absence of an ATP pool. The mean ± standard error for the ATP concentration in the erythrocytes of the four animals was approximately 0.03 ± 0.01 μmole per milliliter of cells, which is two orders of magnitude less than that in human cells. Thus, our results corroborate and extend the observation of McManus (*12*). Moreover, the lack of any ultraviolet-absorbing compound, as determined from the chromatogram, suggests that no other energy-rich pyridine and purine compound is present in the echidna cells. This apparent deficiency of a free energy source may be common to the monotremes, since the erythrocytes of the platypus also exhibit a low ATP content (*13*).

This seemingly implausible energetics notwithstanding, the erythrocytes of the echidna are endowed with an abundance of 2,3-diphosphoglycerate, the average amount from three animals being 5.89 μmole per milliliter of erythrocytes, or 78 percent of the total acid-soluble phosphate content of the cells. The concentration of 2,3-diphosphoglycerate in the echidna cell is comparable to that in human and other mammalian cells. Other phosphorylated compounds are also present and the presence of hexosemonophosphates has been confirmed by the anthrone and carbazole test (*16*). Although several compounds eluting between hexosemonophosphate and 2,3-diphosphoglycerate have not been clearly identified, their elution positions suggest that they might be glucose or mannose diphosphates and monophosphoglyceric acid.

The ATP concentration we observed may represent an upper limit. Although the usual care was taken to remove the buffy coat during the cell preparation, it is possible that white cell contamination contributed to the ATP pool. It is now a considerable challenge to understand the mechanism by which the echidna erythrocytes, having only a trace of ATP, can sustain normal cellular integrity. The likelihood of a substantial free energy requirement appears to be strengthened by the measurement of cation distribution across the cell membranes. The echidna red blood cells have a potassium content of 62.7 mmole per liter of cells, which represents a more than 18-fold concentration gradient of potassium ions across the cell membranes (data not shown). This implies a possible operation of an active cation transport system. Moreover, the large amounts of 2,3-diphosphoglycerate and other phosphorylated compounds suggest a metabolic pathway permitting glycolytic carbon flow. How the glycolytic pathway can be energized is difficult to explain at this time.

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References and Notes

1. R. P. Watts, K. Brendel, M. G. Luthra, H. D. Kim, *Life Sci.* **25**, 1577 (1979).
2. H. D. Kim, R. P. Watts, M. G. Luthra, C. R. Schwalbe, R. T. Conner, K. Brendel, *Biochim. Biophys. Acta* **589**, 256 (1980).
3. S. M. Jarvis, J. D. Young, M. Ansay, A. L. Archibald, R. A. Harkness, R. J. Simmonds, *ibid.* **597**, 183 (1980).
4. M. Nakao, T. Nakao, S. Yamazoe, *Nature (London)* **187**, 945 (1960).
5. ———, H. Yoshikawa, *J. Biochem.* **49**, 487 (1961).

6. D. C. Tosteson and J. F. Hoffman, *J. Gen. Physiol.* **44**, 169 (1960).
7. A. Chanutin and R. R. Curnish, *Arch. Biochem. Biophys.* **121**, 96 (1967).
8. K. V. Kaloustian and J. J. Poluhowich, *Comp. Biochem. Physiol.* **53A**, 245 (1976).
9. H. D. Kim, *Biochim. Biophys. Acta* **588**, 44 (1979).
10. S. Rapoport and G. M. Guest, *J. Biol. Chem.* **138**, 269 (1941).
11. T. J. McManus, *The Human Red Cell in Vitro*, T. J. Greenwalt and G. A. Jamieson, Eds. (Grune & Stratton, New York, 1974), p. 49.
12. ———, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **26**, 1821 (1967).
13. R. E. Isaacks, J. D. Sallis, S. C. Nicol, R. B. Zeidler, H. D. Kim, in preparation.
14. S. P. Bessman, *Anal. Biochem.* **59**, 524 (1974).
15. ———, P. J. Geiger, T. C. Lu, E. R. B. McCabe, *ibid.*, p. 533.
16. R. E. Isaacks, D. R. Harkness, G. A. Froeman, S. A. Sussman, *Comp. Biochem. Physiol* **53A**, 95 (1976).
17. A preliminary account of these findings was presented at the 72nd annual meeting of the American Society of Biological Chemists, St. Louis, Mo., 31 May 1981. Supported in part by NIH grants AM 27570 (to H.D.K.), AM 20815 (to R.E.I.), and research career development award AM 00820 (to H.D.K.), and in part by NSF grant PCM 7815821, which was originally funded in connection with the 1980 *Alpha Helix* expedition to Australia. We thank Drs. B. Johnson, A. F. Cobbold, E. S. Holdsworth, and E. Guiler for the use of facilities at the University of Tasmania, Hobart, Tasmania, and I. Eberhard of the National Parks and Wildlife Service of Tasmania for permission to work on the echidna.

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Cilium Length: Influence on Neural Tonotopic Organization

Abstract. *Previous studies have suggested that variations in the length of the hair cell cilia contribute to auditory nerve fiber tuning and tonotopic organization. In the granite spiny lizard, cilium length and the tonotopic organization of nerve fibers are correlated: fiber characteristic frequency increases as cilium length decreases. This results in an increasing fiber characteristic frequency in both the apical and basal direction, a pattern not previously seen in any vertebrate.*

In the mammalian auditory system, acoustic stimulation causes the basilar membrane to vibrate. The membrane is mechanically tuned in that a particular frequency will cause maximum vibration at one location on the membrane, with the basal portion of the membrane responding best to high frequencies and the apical portion to low frequencies. Membrane motion is transduced into neural excitation by the hair cells, the cochlear sensory receptors, which are located in the organ of Corti and situated on the basilar membrane. Vibration of the basilar membrane causes displacement of the hair cell cilia. This results in changes in the hair cell receptor potentials and the activity of primary auditory nerve fibers. Individual fibers are tuned —that is, they are most sensitive to a certain stimulus frequency (characteristic frequency). Fibers of different characteristic frequencies (CF's) display a tonotopic organization: fibers with high CF's innervate the basal region of the cochlea, whereas those with low CF's innervate the apical region. In the mammal, tonotopic organization of the nerve is consistent with the mechanical tuning of the basilar membrane.

For the mammal, it is accepted that the mechanical tuning of the basilar membrane contributes to neural tuning and tonotopic organization. For many years, however, there has been a controversy as to the need for additional mechanisms. Recent data on hair cell receptor potentials and basilar membrane mechanics suggest that other mechanisms, located at the hair cells, may also contribute to fiber tuning and tonotopic organization (*1*, *2*). In addition, measurements of basilar membrane motion in the alligator lizard indicate that tuning of the basilar membrane does not contribute significantly to neural tonotopic organization; other mechanisms must be present in that animal (*3*, *4*).

Systematic variations in the length of hair cell cilia have been observed in several animals. This variation may contribute to neural tonotopic organization (*3*, *5*). In the chinchilla, cilium length systematically decreases in the basal direction along the cochlea (*5*). This has also been observed in two nonmammalian vertebrates—the chick (*6*) and the alligator lizard (*3*). In the chinchilla and the alligator lizard, where neural tonotopic organization is known, there is a correlation between decreasing cilium length and increasing CF. However, the causal effects of cilium length are difficult to separate from position on the basilar membrane.

The ear of the granite spiny lizard (*Scelopolus orcutti*, family Iguanidae) provides an excellent opportunity to investigate the relation between cilium

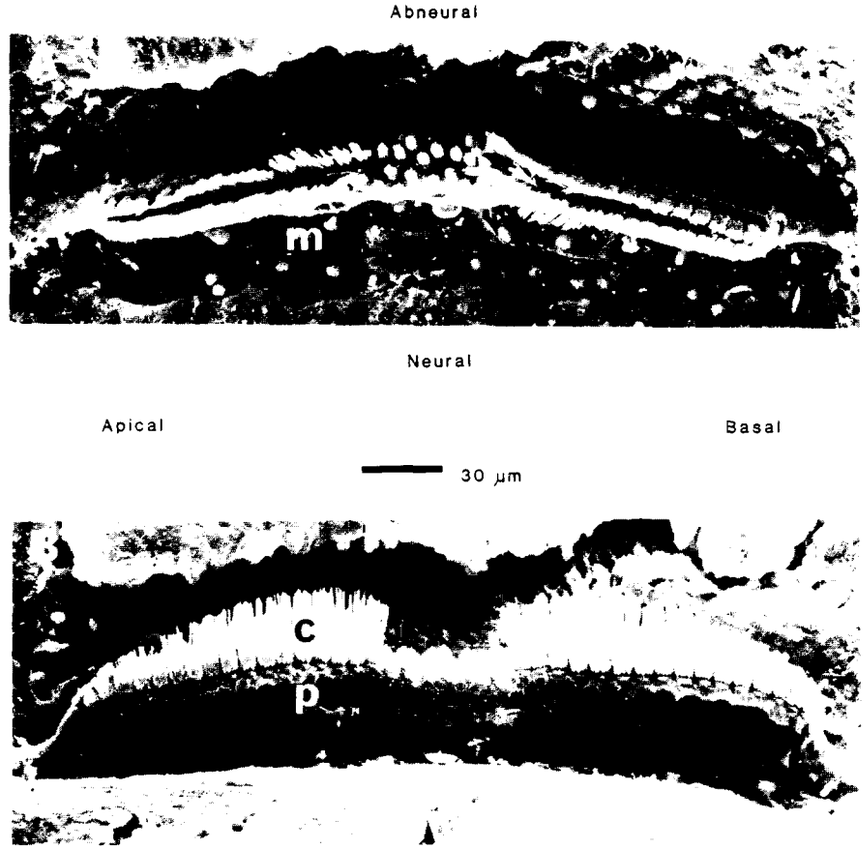

Fig. 1. Scanning electron micrographs of the right basilar papilla (*p*) of the granite spiny lizard. Note the three distinct hair cell populations in the top (A) and side (B) views. The central population has shorter cilia and a tectorial membrane (not visible). Both the apical and basal populations have longer free-standing cilia (*c*) which decrease systematically in length along the papilla. The basilar membrane (*m*) is evident in the top view (A). The membrane does not decrease systematically in width in the basal direction. Neural means toward the auditory nerve; abneural, away from the nerve.

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