High Macromolecular Synthesis with Low Metabolic Cost in Antarctic Sea Urchin Embryos

Adam G. Marsh,[1]* Robert E. Maxson Jr.,[2] Donal T. Manahan[1]†

Assessing the energy costs of development in extreme environments is important for understanding how organisms can exist at the margins of the biosphere. Macromolecular turnover rates of RNA and protein were measured at −1.5°C during early development of an Antarctic sea urchin. Contrary to expectations of low synthesis with low metabolism at low temperatures, protein and RNA synthesis rates exhibited temperature compensation and were equivalent to rates in temperate sea urchin embryos. High protein metabolism with a low metabolic rate is energetically possible in this Antarctic sea urchin because the energy cost of protein turnover, 0.45 joules per milligram of protein, is 1/25th the values reported for other animals.

In the cold waters of Antarctica, embryos and larvae of marine invertebrates have extended developmental periods (several months to a year) and low metabolic rates (*1*). The effects of limited food and cold temperature are considered major selective forces resulting in slow developmental processes in polar seas (*2*). The relation between development, macromolecular synthesis, and energy utilization is critical for understanding the physiological mechanisms that determine larval survival in species inhabiting these extreme environments. Protein biosynthesis is a major determinant of an organism's total energy budget (about 30%) (*3*), and studies of temperature effects on metabolism have led to the suggestion that changes in protein turnover are an evolutionary component of metabolic temperature adaptation (*4*).

We measured protein synthesis and turnover during development of the Antarctic sea urchin *Sterechinus neumayeri* at −1.5°C by quantifying rates of incorporation of [^{14}C]alanine and [^{14}C]leucine into protein. High-performance liquid chromatography was used to measure free amino acid pools of alanine and leucine for calculations of the intracellular specific activity (*5*, *6*). Gas-phase acid hydrolysis was used to quantify the mole percent of alanine (7.0% ± 0.2 SD) and leucine (10.8% ± 0.3 SD) in proteins of *S. neumayeri* for calculations of the total mass of synthesized protein from the amino acid incorporation rates (*7*).

Metabolic rates for embryos of *S. neumayeri* were low, increasing from only 2 to 12 pmol O_2 hour^{-1} per individual during development to the four-arm, pluteus larval stage (22 days of development) (*8*). Once this pluteus larval stage was reached, respiration rates stabilized between 13 and 16 pmol O_2 hour^{-1} per individual to day 47 (the time period studied). Protein synthesis rates were about 5 ng hour^{-1} except during gastrulation (day 12), when a rapid increase in protein synthesis was evident (15 to 25 ng hour^{-1}; Fig. 1A) (*9*). Once the pluteus larval stage was reached (day 22), further development to day 50 resulted in a decline in protein synthesis rates to 0.2 to 0.7 ng hour^{-1}. Measurements of protein synthesis using both alanine and leucine as tracers were equivalent.

During embryogenesis and early development of unfed larvae of *S. neumayeri*, measured rates of protein synthesis were equivalent to rates of protein turnover, because there was no increase in protein mass of the embryos or larvae (*10*). The fractional rates of protein turnover for *S. neumayeri* were calculated by expressing rates of synthesis as a percentage of total protein content during development. These fractional rates (percentage of total body protein turned over per hour) were 2.2% for blastulae (*11*), increased during gastrulation (10% per hour), and were then low in pluteus larvae (<1% per hour). Reports of protein turnover for blastula stages of some well-studied temperate species of sea urchins provide data for a comparison of fractional rates of protein turnover (percent per hour): *S. neumayeri*, 2.2% (−1.5°C); *Strongylocentrotus purpuratus*, 1.1% (16°C) (*12*); *Lytechinus pictus*, 1.0% (19°C) (*13*); and *Arbacia punctulata*, 1.9% (25°C) (*14*). These rates were equivalent despite large differences in environmental temperatures. The fractional rate of protein turnover in embryos of *S. neumayeri* at −1.5°C exhibits temperature compensation (2% per hour), despite a thermal gradient that should theoretically result in a physiological rate reduction in protein turnover to one-fourth to one-sixth of the values shown for temperate sea urchin embryos (using a conservative $Q_{10} = 2$, a common parameter used to adjust physiological rate measurements between different temperatures; Fig. 1B). We conclude that embryos of *S. neumayeri* demonstrate full temperature compensation for rates of protein turnover.

To address a potential mechanism for the high protein turnover rates at −1.5°C , we measured the amount of whole-cell RNA and poly(A$^+$) RNA in eggs and embryos of *S. neumayeri* and, for comparison, in *S. purpuratus*. Both whole-cell RNA and poly(A$^+$) RNA fractions were substantially higher in hatching

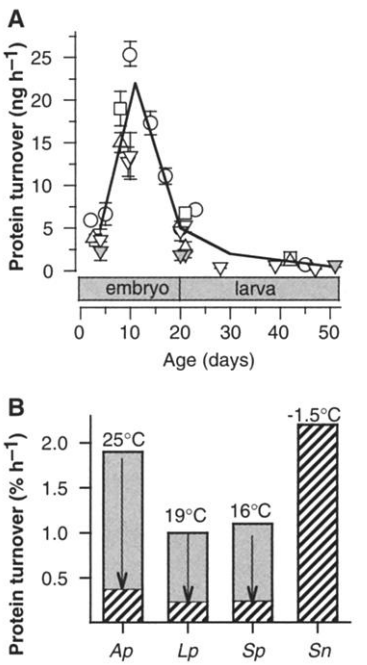

Fig. 1. Protein turnover rates during development of *S. neumayeri*. (**A**) Protein turnover rates (that is, synthesis rates in embryos and larvae of *S. neumayeri*) during development were calculated from the free amino acid specific activity of a radiolabel, the mole percent amino acid composition, and the rates of labeled amino acid incorporation into protein. Open symbols represent four different cultures for which alanine was used as the radiolabeled tracer; leucine was also used for corroboration of rates (gray triangles) on one of those cultures. All points are plotted as means ± 1 SEM ($n = 5$). (**B**) Fractional rates of protein turnover (per unit egg protein mass; percent per hour) for different species of sea urchin embryos at the blastula stage. The temperatures at which these measurements were made are indicated above the solid bars. The hatch bars indicate the turnover rates at −1.5°C as directly measured in *S. neumayeri* (*Sn*) and estimated by Q_{10} extrapolations (using a conservative value of 2.0) in the temperate urchins *Arbacia punctulata* (*Ap*), *Lytechinus pictus* (*Lp*), and *Strongylocentrotus purpuratus* (*Sp*).

[1]Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, USA. [2]Department of Biochemistry and Molecular Biology, USC/Norris Hospital Institute, University of Southern California School of Medicine, Los Angeles, CA 90033, USA.

*Present address: College of Marine Studies, University of Delaware, Lewes, DE 19958, USA.
†To whom correspondence should be addressed. E-mail: Manahan@usc.edu

blastulae of *S. neumayeri* than those of *S. purpuratus*. Whereas hatching blastulae of *S. purpuratus* contain a constant 3 ng of whole-cell RNA (*15*) and 69 ± 1.2 pg of poly(A^+) RNA (our data) during early development, *S. neumayeri* embryos show a large increase in poly(A^+) RNA levels (Fig. 2A), and at the blastulae stage, they contain as much as 115 ± 11 ng of whole-cell and 8165 ± 441 pg of poly(A^+) RNA (per embryo). Normalized to embryo volume, *S. neumayeri* had approximately 10 times the cellular concentration of poly(A^+) RNA (2.67 fg mRNA μm^{-3}) as did *S. purpuratus* (0.25 fg mRNA μm^{-3}) (*15*). Elevated levels of poly(A^+) RNA and whole-cell RNA may in part explain the mechanistic basis for the high rates of protein turnover in embryos of *S. neumayeri*.

Greater levels of poly(A^+) RNA in *S. neumayeri* may result from increased synthesis rates and/or reduced degradation rates. Measurements of absolute rates of synthesis of total RNA in *S. neumayeri* (picograms RNA per hour per cell) evidenced temperature compensation and were equivalent to rates in *S. purpuratus* (*15*) and *L. pictus* (*16*), despite large differences in environmental temperatures (Fig. 2B). Even more noteworthy, the synthesis rates of whole-cell poly(A^+) mRNA were much higher in *S. neumayeri* (0.14 pg mRNA $hour^{-1}$ per cell) when compared with embryos of *L. pictus* and *S. purpuratus* (0.04 and 0.03 pg mRNA $hour^{-1}$ per cell, respectively), even without a temperature correction (Fig. 2C). In addition, the percentage of total RNA synthesis allocated to mRNA production was higher in *S. neumayeri* (42% versus 9% of total RNA synthesis; compare parts B and C of Fig. 2). The turnover of poly(A^+) mRNA was calculated from a kinetic analysis of the radiolabel incorporation rates (*17*) and revealed a half-life of 4.1 hours in *S. neumayeri*. A similar analysis for *L. pictus* from published data (*16*) produced a near-equivalent turnover time of 4.3 hours. Thus, the 10-fold elevation of poly(A^+) mRNA per unit volume in *S. neumayeri* relative to *S. purpuratus* (Fig. 2A) most likely results from an increased synthesis rate of mRNA and not a reduced degradation rate at low temperatures.

High rates of macromolecular turnover with low rates of respiration in *S. neumayeri* pose a physiological paradox: a 2% per hour protein turnover rate would be energetically impossible if metabolic energy costs of protein metabolism were the same at –1.5°C as has been reported in other animals. The aerobic cost of protein turnover can be estimated from a linear regression of total respiration rates against protein synthesis rates, an approach used routinely in metabolic studies (*18*). This cost includes the energy utilization of all cellular activities involved in protein turnover, including RNA synthesis and processing. For late embryos and larval stages of *S. neumayeri* (after gastrulation, day 17 to day 47) the regression revealed a low energy cost of protein turnover (Fig. 3A). The slope of this regression line was 0.94 (± 0.14 SE) pmol O_2 $hour^{-1}$ respired for 1 ng $hour^{-1}$ of protein turnover ($r^2 = 0.802$, $n = 13$), which is equivalent to 0.45 J mg^{-1} protein (at 484 kJ mol^{-1} O_2). A synthesis cost of 0.45 J mg^{-1} protein in *S. neumayeri* is about 1/25th of values reported for a variety of other animals calculated by using a similar approach [e.g., the mussel *Mytilus edulis*, 11.4 J mg^{-1} (*19*); a cod fish, 8.7 J mg^{-1} (*20*); mammals, 12.6 J mg^{-1} (*21*)].

Using a net energy cost of 0.45 J mg^{-1} protein for *S. neumayeri*, protein synthesis rates (Fig. 1A) can be converted to the fraction of total energy metabolism consumed by protein turnover during development. The contribution of protein turnover to total metabolic demand was 53% at the hatching blastula stage (Fig. 3B). The in vivo metabolic energy consumption of Na^+/K^+-ATPase (the sodium ion pump, another major component of cellular energy metabolism in animals) has been measured during development in *S. neumayeri* (*10*). For a hatching blastula, protein turnover and sodium ion regulation account for 65% of total metabolism (Fig. 3B), which is consistent with what is known about metabolic energy partitioning in other animals

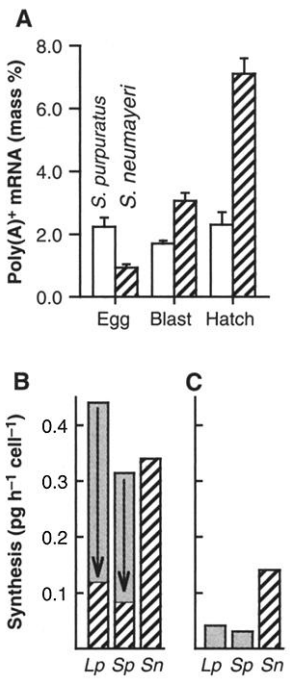

Fig. 2. Amount of RNA and rates of synthesis in *S. neumayeri*. (**A**) Amounts of poly(A^+) mRNA are presented for *S. neumayeri* and *S. purpuratus* (mean ± 1 SD) during early development for a zygote (egg), and blastula (blast) and hatching blastula (hatch) stages. (**B**) Synthesis rates of whole-cell, total RNA (picograms per hour per cell) for different species of sea urchin embryos at the blastula stage. The temperatures at which these measurements were made are given below. The hatched bars indicate the synthesis rates at –1.5°C as directly measured in *S. neumayeri* (*Sn*, –1.5°C) and estimated by Q_{10} calculations (using a conservative value of 2.0) in the temperate urchins *Lytechinus pictus* (*Lp*, 19°C) and *S. purpuratus* (*Sp*, 16°C) [from published values (*13, 14*)]. (**C**) Synthesis rates of whole-cell, poly(A^+) mRNA (mRNA, picograms per hour per cell) for different species of sea urchin embryos at the blastula stage (*Lp*, 19°C; *Sp*, 16°C). Errors for the *S. neumayeri* synthesis rates in (B) and (C) were calculated but are not shown on the graph—the standard error of the regression coefficients that were used to calculate these net synthesis rates was approximately ± 20%.

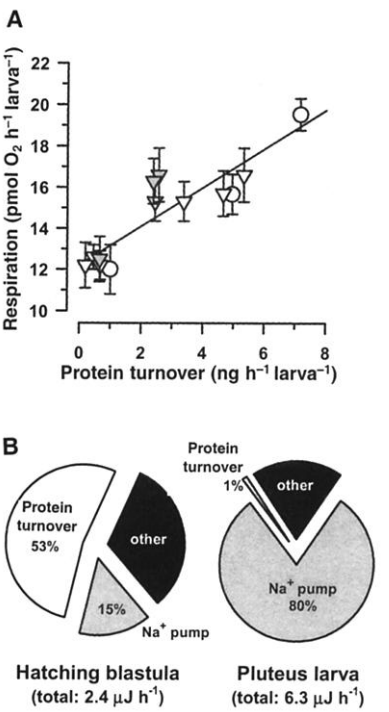

Fig. 3. Energetics of protein metabolism in embryos and larvae of *S. neumayeri*. (**A**) The energy costs of protein turnover rates during development of *S. neumayeri* were calculated from a regression of respiration and protein synthesis rates. A linear relation between respiration and protein synthesis was evident after day 17 of development, the transition from a late prism-stage embryo to early pluteus-stage larva (see Fig. 1). Symbols correspond to the same cultures as in Fig. 1. Protein turnover was measured in larvae with both alanine (open symbols) and leucine (gray triangles) as the radiolabeled amino acid tracers. The data (n = 13) include some previously measured respiration rates [(open triangles (27)]; other symbols represent independent measurements made on additional cultures. (**B**) The fraction of total metabolic energy expenditure accounted for by protein metabolism in *S. neumayeri*. Energy consumed by protein metabolism was calculated from the ratio of respiration to protein turnover [0.45 J mg^{-1} protein; (A)] and then expressed as a percentage of total metabolic energy expenditure. Energy consumption of the Na^+ pump has been published elsewhere (*10*).

(*22*). Once a larval stage is reached (day 22), protein metabolism had decreased to 30% of total metabolism (*23*) and then further declined to 1% for a larva at day 50 (Fig. 3B, right-side of pie chart). At this point in larval development, the sodium pump consumes a very large fraction of total metabolic energy (80%) (*10*), and a reduction in the cost of protein turnover is necessary to accommodate the sodium pump's demand for cellular energy, given the low metabolic rates of these embryos and larvae.

A relative increase in the rates of mRNA synthesis and protein turnover at −1.5°C is energetically possible in *S. neumayeri*, because the cost of protein metabolism is very low. Indeed, the value we report is lower than has been reported for any other animal. The increase in poly(A^+) mRNA synthesis can provide a proximate explanation for the unexpectedly high rate of protein turnover in this Antarctic animal. The thermodynamic bases remain to be elucidated for such energy efficiency of protein turnover at low temperatures. Further analyses of the processes underlying the greater energy efficiency in protein metabolism may uncover novel mechanisms of biochemical adaptations and lead to a better understanding of metabolic diversity in organisms inhabiting extreme polar environments.

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6. Early work on protein synthesis rates in sea urchin embryos has demonstrated a rapid equilibration between the intracellular free amino acid pool and the charged tRNA pool (*24*).
7. Amino acid composition of proteins was examined during development in *S. neumayeri* by gas-phase acid hydrolysis and high-performance liquid chromatography quantification of constituent amino acids (*5, 25*). A trichloroacetic acid precipitation step was used to isolate protein from whole embryos and larvae for the analyses. Seven different developmental time points were measured between fertilization and the first larval stage (day 22).
8. Respiration rates were measured using 1-ml glass respiration vials for end-point measurements of oxygen tension using a polarographic oxygen sensor (*26, 27*). Six vials with 50 to 200 embryos or larvae were measured at each experimental point by sealing the vials and incubating them for 8 hours at −1.5°C. Oxygen tension was then measured by injecting 500 μl of the seawater from a vial into a 50-μl microcell maintained at −1.5°C. This technique was specifically optimized for use with *S. neumayeri* embryos and larvae and cross-checked with four other independent methods for measuring oxygen consumption rates.
9. Protein synthesis rates were measured during 90-min time-course experiments of radiolabel incorporation into trichloroacetic acid–precipitable protein (*28*). During embryogenesis, rates were measured on four separate cultures (started from different parents at different times), and two of these were further used for studies during later larval development. For all experiments, 12,000 individuals were placed in 12 ml of sterile filtered seawater (0.2 μm) with 70 μCi of [^{14}C]alanine. Individuals were incubated at −1.5°C with the radiolabel, and 500-μl aliquots were removed at 15-min intervals for measurements of both the protein incorporation and the specific activity of [^{14}C]alanine in the free amino acid pool. Corroborative measurements were also made using [^{14}C]leucine.
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A Short Duration of the Cretaceous-Tertiary Boundary Event: Evidence from Extraterrestrial Helium-3

S. Mukhopadhyay,[1]* K. A. Farley,[1] A. Montanari[2]

Analyses of marine carbonates through the interval 63.9 to 65.4 million years ago indicate a near-constant flux of extraterrestrial helium-3, a tracer of the accretion rate of interplanetary dust to Earth. This observation indicates that the bolide associated with the Cretaceous-Tertiary (K-T) extinction event was not accompanied by enhanced solar system dustiness and so could not have been a member of a comet shower. The use of helium-3 as a constant-flux proxy of sedimentation rate implies deposition of the K-T boundary clay in $(10 \pm 2) \times 10^3$ years, precluding the possibility of a long hiatus at the boundary and requiring extremely rapid faunal turnover.

The K-T boundary at 65 million years ago (Ma) records a major mass-extinction event and, though the occurrence of an extraterrestrial impact (*1*, *2*) is widely accepted, the nature of the impactor and its role in the K-T mass extinction is debated. Possible candidates for the impactor are a single asteroid or comet (*1–3*) or a member of a comet shower (*4*). An extraterrestrial impact would have severely perturbed Earth's ecosystems and climate by injecting large quantities of dust (*1*) and climatically active gases (*5*) into the atmosphere. An alternative hypothesis to explain the biotic calamity invokes voluminous volcanism (*6*). Recent work (*7*) suggests that most of the Deccan Traps flood basalts were erupted in a <1-million-year (My) interval coincident with the K-T boundary. The global environmental effects from extensive volcanism could be similar to the effects from a large impact (*6*), but the time scale of the two processes would be different. The perturbation on climate and ecosystems from an impact would be geologically instantaneous, but the effects from volcanism would be spread over at least a few hundred thousand years.

The K-T boundary clay is a distinctive bed, typically a few cm thick, that separates sedimentary rocks of the Cretaceous from those of the Tertiary. Knowledge of the deposition interval of the clay would provide important insights into the cause(s) and rates of mass extinction and climate change at the boundary, but most geochronologic tools are inadequate for this purpose. Estimates of this time interval are based on the assumption that the K-T clay was deposited at the same rate

[1]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA. [2]Osservatorio Geologico di Coldigioco, 62020 Frontale di Apiro, Italy.

*To whom correspondence should be addressed. E-mail: sujoy@gps.caltech.edu