3. Y. Termonia, *Macromolecules* **27**, 7378 (1994).
4. J. T. Prince, K. P. McGrath, C. M. DiGirolamo, D. L. Kaplan, *Biochemistry* **34**, 10879 (1995).
5. M. J. Fournier, T. L. Mason, D. A. Tirrell, in *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, R. A. Meyers, Ed. (VCH Publ., New York, 1995), p. 714; M. T. Krejchi *et al.*, *Science* **265**, 1427 (1994); D. Byrom, Ed., *Biomaterials* (Stockton, New York, 1991).
6. J. M. Gosline, M. E. DeMont, M. W. Denny, *Endeavor* **10**, 37 (1986).

Ecdysis Control Sheds Another Layer

James W. Truman

The dramatic escape of a butterfly from its chrysalis is one of the most commonly photographed behavioral sequences in biology. The apparent abruptness of this ecdysis, however, is misleading because the transformation actually takes days or weeks but is hidden from sight within the cuticle of the chrysalis. The steroid hormone ecdysone and its metabolites cause the epidermis of the chrysalis to detach from its overlying cuticle and begin to form the butterfly. As this molting process progresses, the inner layers of the old cuticle are digested, leaving it vulnerable to the forces that will eventually cause it to rupture. The forming new cuticle is soft and pliable, a condition that allows it to be slipped out from the confining sheath of the old cuticle. Only when the developmental events are complete are the ecdysial processes then called into play to bring about the shedding of the old cuticle and the expansion and hardening of the new one. The triggering of ecdysis itself is hormonally controlled, in a process that just became more complex. In this issue, Žitňan and colleagues report the existence of a second ecdysial hormone—Mas-ETH (*Manduca sexta* ecdysis-triggering hormone)—to add to the existing eclosion hormone (*1*).

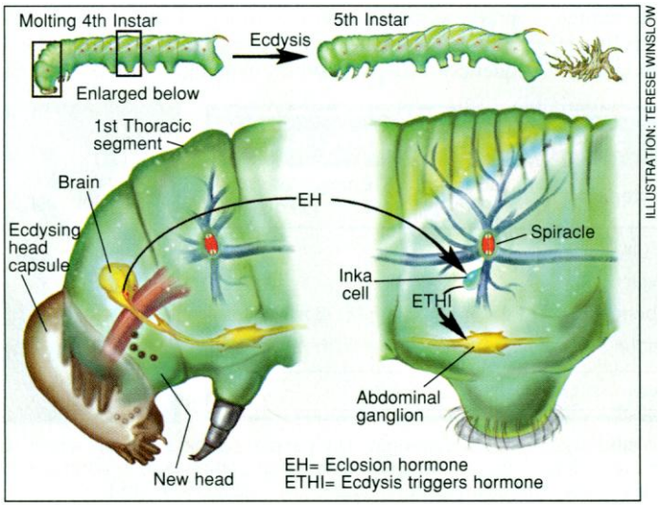


ILLUSTRATION: TERESE WINSLOW

Mechanism of ecdysis control. The pathway for triggering ecdysis behavior originates in the brain but is relayed through the Inka cells, a set of glands in the periphery.

Although in a sense a conjurer's illusion, ecdysis is nevertheless a process worthy of fascination. It includes a sequence of complex behavioral programs that enable the insect to shed its entire body surface, including the cuticular linings of its fore- and hindgut and tracheal system (*2*). Many of the behaviors seen during ecdysis are unique to this event and, indeed, after the final molt to the adult stage, the neurons dedicated to these behaviors then die (*3*). A successful ecdysis requires that these specialized behaviors be closely coordinated with ongoing physiological and developmental processes (*2*). Because of irreversible changes within the new cuticle, the behavior can only be performed once. Hence, a failure in this coordination results in crippling deformities or in insects being fatally trapped in their old skin, and each ecdysis is a potential crisis point in an insect's life history.

The hormonal control of ecdysis was initially inferred from experiments on moths that showed that the circadian timing of this behavior could be transferred from one species to another by transplanting the brain (*4*). These experiments led to the isolation of a peptide from the brain that could induce precocious ecdysis behavior and was called eclosion hormone (EH). In more striking studies in the moth *Hyalophora cecropia*, EH was shown to act on an isolated chain of abdominal ganglia with its accompanying tracheal supply to release the complete preecdysis and ecdysis motor programs (*5*). This 62–amino acid peptide was purified and sequenced from the moths M. *sexta* (*6*, *7*) and *Bombyx mori* (*8*).

The new report by Žitňan *et al.* (*1*) indicates that the long-held view that EH is the sole mediator of ecdysial behavior and physiology is too simplistic. They have found a second peptide from M. *sexta*, an ecdysis-triggering hormone (Mas-ETH), which can also trigger the preecdysis and ecdysis behaviors. This peptide is released from a set of peripheral secretory cells, Inka cells, that are situated on ventral tracheal trunks near each spiracle. When injected into molting animals, the Inka peptide induces the rapid onset of preecdysis and ecdysis behaviors. The sequence of this 26–amino acid peptide is novel and is structurally unrelated to that of EH.

What is the functional relation of Mas-ETH to EH? The authors describe an insightful set of experiments on the isolated central nervous system (CNS) of M. *sexta*. The isolated CNS, without its tracheal supply, does not respond to EH, but it does respond to Mas-ETH by generating both the preecdysis and ecdysis motor programs. The isolated CNS can be made to respond to EH in vitro if freshly dissected Inka cells are also placed in the bath. These findings on *Manduca* call into question the interpretation of the need for the tracheal system in the original studies on the isolated CNS of *H. cecropia*. Ironically, the ventral tracheal trunk that leads to each ganglion is also the one on which the Inka cell resides.

As depicted in the figure, these new results suggest that a peripheral step involving the Inka cells is interposed between the secretion of EH and the subsequent triggering of the ecdysial motor programs from the CNS. Because a successful ecdysis requires that the old cuticle be sufficiently digested to be shed, it seems appropriate that there is peripheral input in deciding the timing of ecdysis. It is especially intriguing that the Inka cells are located on the trachea near each spiracle, the opening through which the tracheal linings are withdrawn. These linings are the most fragile parts of the old cuticle, and if they are torn and left behind during ecdysis, the tracheae to that region are obstructed and oxygen delivery is impaired. Thus, the Inka cells are in an excellent position to monitor the changes in the tracheae during the molt. Sitting at this most vunerable site in the periphery, they might serve as a final checkpoint to ensure that the old cuticle is ready before the insect irreversibly commits itself to ecdysis.

Although the relation depicted in the figure seems likely, some observations remain unexplained. For example, experiments on pupal ecdysis in *Manduca* show that ecdysis still occurs when the secretion

The author is in the Department of Zoology, University of Washington, P.O. Box 351800, Seattle, WA 98195–1800, USA. E-mail: trumanj@zoology.washington.edu

of EH into the circulation is prevented by removal of the peripheral release sites (9). The occurrence of the ecdysial behaviors under these conditions is thought to be due to a local release of EH within the CNS. By contrast, peripheral EH targets, such as dermal glands, do not secrete their products because of the lack of circulating EH. Given the arrangement in the figure, the lack of blood-borne EH should also result in a failure of the Inka cells to release their peptide. Clearly, more work needs to be done to define the exact relation between the release and action of EH and of Mas-ETH. Also, the relation of Inka cell activity to the phases of the molt cycle needs to be explored to determine whether they are involved in assessing the "readiness" of the periphery.

Regardless of these details, the discoveries of the Inka cells and Mas-ETH have opened up an exciting new chapter in the study of the complex process of ecdysis.

References

1. D. Žitňan *et al.*, *Science* **271**, 88 (1996).
2. S. E. Reynolds, *Adv. Insect Physiol.* **15**, 475 (1980).
3. J. W. Truman, S. E. Fahrbach, K.-I. Kimura, *Prog. Brain Res.* **86**, 25 (1990).
4. J. W. Truman and L. M. Riddiford, *Science* **167**, 1624 (1970).
5. J. W. Truman, *J. Exp. Biol.* **74**, 151 (1978).
6. T. Marti, K. Takio, K. A. Walsh, G. Terzi, J. W. Truman, *FEBS Lett.* **219**, 415 (1987).
7. H. Kataoka, R. G. Troestschler, S. J. Kramer, B. J. Cesarin, D. A. Schooley, *Biochem. Biophys. Res. Commun.* **146**, 746 (1987).
8. T. Kono, H. Nagasawa, A. Isogai, H. Fugo, A. Suzuki, *Agric. Biol. Chem.* **51**, 2307 (1987).
9. R. S. Hewes and J. W. Truman, *J. Comp. Physiol. A* **168**, 697 (1991).

Stardust in the Laboratory

Ernst Zinner

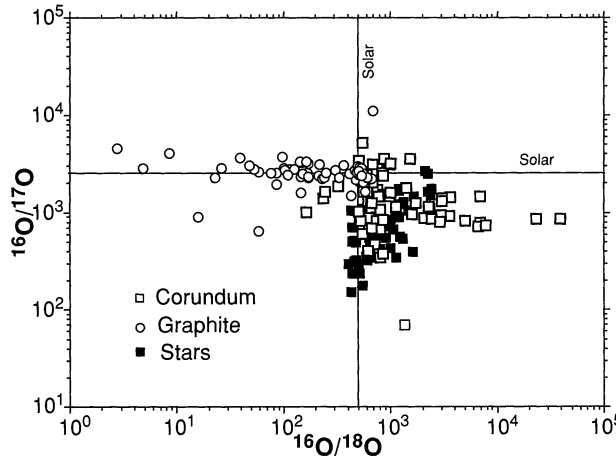
Although the elements from carbon on up are expected to be produced in different stars by a variety of nuclear processes with very different isotopic ratios (*1*), solar system materials—even primitive meteorites, which contain the oldest solar system objects—have uniform isotopic compositions. This uniformity could be the result of extremely thorough mixing of the source material that formed the solar system. How wide the range of isotopic compositions of this material actually is was not realized until a few years ago: Preserved stardust was discovered in meteorites, and individual micrometer-sized stellar grains were isolated and studied in detail in the laboratory (*2*, *3*). These grains are believed to have condensed in stellar outflows and supernova ejecta and thus preserve the elemental and isotopic composition of their stellar sources. The range of their isotopic compositions not only dwarfs that observed in solar system objects but by far exceeds the range of spectroscopic observations in stars (see figure).

Although the presence of stardust in meteorites had already been indicated in the sixties by the presence of "exotic"—that is, isotopically anomalous—noble gas components in different meteorites, it took more than 20 years before the carriers of these noble gases were identified and isolated (*4*). Today, the list of types of stardust include diamond, silicon carbide (SiC), graphite, aluminum oxide (corundum), and silicon nitride [see, for example (*2*, *3*, *5*, *6*)]. In addition, SiC and graphite were found to contain tiny subgrains of titanium, zirconium, and molybdenum carbides, identified in the transmission electron microscope (*7*).

The ion microprobe plays a central role for the analysis of meteoritic stardust because this instrument makes it possible to measure the isotopic compositions of individual grains down to sizes of less than 1 μm. On the basis of the isotopic compositions of the grains, two important types of stellar sources could be identified: red giant stars of low to medium mass during late stages of their evolution, and supernovae, massive stars that exploded at the end of their evolution.

Most of the SiC grains are believed to have originated from red giants, specifically from carbon-rich, thermally pulsing, asymptotic giant branch (AGB) stars (*8*). The AGB stars are believed to be the main source of s-process (nucleosynthesis by slow neutron capture) elements, and "bulk samples" (collections of many grains) of SiC grains carry the s-process signature in the isotopic compositions of Kr, Xe, Ba, Nd, and Sm (*2*). The isotopic compositions of C, N, and Mg measured in most single SiC grains generally agree with a carbon-star origin and can be explained by nucleosynthesis during H and He burning in deep stellar layers and mixing of the products to the surface of stars losing mass in strong stellar winds. The Si and Ti isotopic compositions of single grains (*9*) cannot be explained by nucleosynthesis taking place in a single star and indicate multiple stellar sources (*10*).

Corundum grains show a large range in their O isotopic ratios (see figure) and $^{26}Al/^{27}Al$ ratios (*5*, *11*). Some grains must have formed in the expanding atmosphere of red giants. As in the case of Si isotopic compositions of SiC grains, the O isotopic compositions of individual corundum grains cannot be explained by nucleosynthetic processes taking place in a single star. Excesses in ^{17}O and moderate depletions in ^{18}O relative to the star's original composition are believed to result from mixing into the envelope of material processed in the star's interior during core H burning. However, the spread in O isotopic compositions found in meteoritic oxide grains can only be explained by assuming that different stars with different masses (variations in $^{16}O/^{17}O$) and different initial isotopic compositions (variations in both $^{16}O/^{17}O$ and $^{16}O/^{18}O$) contributed oxide grains to the solar system (*12*). Some grains with high $^{16}O/^{18}O$ ratios (see figure) must have come from AGB stars of intermediate mass in which H burning in deep convective layers destroyed essentially all ^{18}O.



Stardust signatures. Comparison of the oxygen isotopic ratios measured in different types of stars with those measured in individual grains of stardust found in meteorites.

Three types of presolar grains in meteorites are believed to come from supernovae: low-density graphite grains, SiC grains of the rare (1% of all SiC) type X, and even rarer silicon nitride. The ^{15}N and ^{18}O excesses found in many of these grains are

The author is at the McDonnell Center for the Space Sciences, Physics Department, and Department of Earth and Planetary Sciences, Washington University, St. Louis, MO 63130, USA. E-mail: ekz@howdy.wustl.edu