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17 September 1976; revised 8 February 1977

Temporal Control of Urate Oxidase Activity in *Drosophila*: Evidence of an Autonomous Timer in Malpighian Tubules

Abstract. *The appearance of urate oxidase activity in the Malpighian tubules of* Drosophila melanogaster *is synchronized with the time of emergence of the imago from the puparium. A developmental clock within the Malpighian tubules specifies the time of appearance of urate oxidase activity.*

Different cell types exhibit their repertoire of proteins in a definite sequence and at a characteristic time during development. A description of the events which control the time of appearance and disappearance of tissue- and stage-specific proteins is of fundamental importance for an understanding of the mechanisms giving rise to the differentiated state.

At least two types of phenomena may be involved in the temporal control of a tissue-specific protein. The phenotype of a cell may be modified by signals emitted from closely apposed or noncontiguous cells at a fixed time during development (*1*). Conversely, an intracellular clocklike mechanism may specify the time of occurrence of a change in the differentiated state. Clocklike phenomena during development have been referred to in discussions of programmed cell death (*2, 3*), aging (*3, 4*), the differentiation of distal wing structures from the progress zone (*5*), and the appearance of an enzyme in mouse trophoblast cells in vitro (*6*).

Mechanisms have been proposed by which a cell might autonomously control the timing of developmental events. These include an intracellular counter for the number of DNA replications or cell divisions (*3, 4, 7*), an accumulation of substances to a threshold level (*8*), or a causally connected progression of intracellular reactions which subsequently activate transcription of a specific genetic locus (*9*). In addition to the above, Bonner (*10*) has speculated about the possible role of free-running endogenous oscillators in the timing of nonperiodic developmental events.

This investigation of the mechanism specifying the time of appearance of urate oxidase activity in the Malpighian tubules of *Drosophila melanogaster* was initiated to evaluate experimentally the role of autonomous biological clocks and humoral factors in the temporal control of a discrete tissue-specific biochemical event. Urate oxidase (E.C. 1.7.3.3) catalyzes the conversion of uric acid to allantoin and, in *Drosophila*, is located exclusively in the Malpighian tubules (*11*), a tissue that does not undergo cell division or reorganization after the larval stage. Urate oxidase activity is not detectable during embryonic development, in first and second instar larvae, or in pupae, but is present in third instar larvae and adults (*11, 12*). Our data indicate that the appearance of urate oxidase activity in the tubules upon emergence of the adult from the puparium is controlled by a developmental clock within the Malpighian tubules (*13*).

The appearance of urate oxidase activity upon emergence is most easily detected when there is a large burst of activity. Therefore, in all experiments we employed a xanthine dehydrogenase–deficient strain (*14*) which has five- to tenfold more urate oxidase activity than wild-type *Drosophila* when measured 1 day after emergence (*11*).

The *Drosophila* were raised at 25°C in constant light on cornmeal, molasses, and yeast food (*15*). Constant light was used because this condition extinguishes the rhythmic pattern of emergence (*16*) which then occurs without delay when development of the imago is complete.

The time remaining until emergence of an adult from the puparium is determined by selecting pupae with yellow, orange, and light brown eyes. These pupae are heterogeneous with respect to the time remaining until emergence which ranges from 37 to 50 hours at 25°C (*17*). Ten hours after the pupae with yellow, orange, and light brown eyes are collected, pupae with red eyes and very light gray wings are selected at hourly intervals. This second step assures that the pupae are viable and synchronized at the same developmental stage. The light gray wing stage lasts approximately 1 hour. Ninety-eight percent of xanthine dehydrogenase–deficient pupae with red eyes and gray wings emerge as adults after 26.5 ± 3 hours when incubated at 25°C.

Malpighian tubules were dissected in *Drosophila* Ringer solution, *p*H 6.9 (*18*), gently aspirated into a glass transplant needle, and inserted into the abdomen of a host. The transplant procedure for *Drosophila* tissues is described by Ursprung (*19*). A pair of anterior tubules and a pair of posterior tubules each connected to their respective collecting ducts were separately transplanted into different hosts anesthetized with ether. After transplantation the hosts were maintained on petri dishes (20 by 100 cm) containing a moistened Kimwipe and a dish (2.2 by 0.7 cm) containing 5 percent dextrose and 1 percent agar. This procedure allowed for easy retrieval of a host after the petri dish was cooled. For the first few days after emergence the presence or absence of food does not influence the level of urate oxidase activity in xanthine dehydrogenase–deficient strains (*11*).

To retrieve the host tubules and transplanted donor tubules, the hosts were submerged and dissected in 0.25*M* sucrose containing 1.7 m*M* EDTA, *p*H 6.9, at 22° to 25°C. After the abdomen was opened, the donor tubules usually floated free and could always be distinguished from the host's tubules which were attached to the alimentary canal. A pair of posterior and anterior donor tubules and one complete set of host tubules were then separately assayed for urate oxidase activity.

Modifications of a radiochemical assay for urate oxidase previously reported (*20*) were used for the detection of activity in the Malpighian tubules from a single *Drosophila*. One complete set of tubules was rapidly transferred on the tip of a dissecting needle to a 1.5-ml Brinkmann test tube. The assay tubes contained 11.0 m*M* boric acid, 1.4 m*M* EDTA (disodium salt), and 0.18 percent Triton X-100 in a total volume of 15 μl at *p*H 9.05. The tubule was incubated in the assay mixture for 20 minutes at 0° to 4°C. This process disrupted the cells and released urate oxidase into the incubation medium. At the completion of the incubation period, 10 μl of 0.59 m*M* (10 mg/100 ml) [2-^{14}C]uric acid (50 to 53 mc/mmole; Amersham/Searle) was added to initiate the reaction carried out at 26°C. After 2 minutes and again at 6 minutes, a 5-μl sample of the reaction mixture was removed and spotted on one of ten sites which were 2.0 cm apart and 1.5

cm from the bottom of a MN-300 polyethyleneimine-impregnated cellulose thin-layer chromatography plate (20 by 10 cm). The [^{14}C]allantoin was then separated from the [2-^{14}C]urate by thin-layer chromatography and the amount of [^{14}C]allantoin formed was counted in a liquid scintillation spectrometer (*20*).

In the first set of experiments Malpighian tubules were removed from pupae 24 hours (± 3 hours) prior to the expected time of emergence of the adult and transplanted into the abdomen of newly emerged female adults without disturbing the host Malpighian tubules. At the time of transplantation, both the host and donor tubules had little or no urate oxidase activity when measured individually with our ultrasensitive assay (Fig. 1). The data in Fig. 1, A and D, indicate that both the host tubules and donor tubules exhibit their scheduled increase in urate oxidase activity. All host tubules showed an immediate increase in urate oxidase activity after transplantation (Fig. 1A), while the donor tubules (Fig. 1D) had no detectable urate oxidase activity during the first 20 hours after transplantation. Forty-eight hours after transplantation, the donor tubules had considerable urate oxidase activity (Fig. 1D).

When a tubule from a pupa with only 10 hours (± 3 hours) of development remaining until emergence was transplanted into the abdomen of a newly emerged adult, the delay in the expression of urate oxidase activity was approximately 7 hours (Fig. 1C). The transplanted tubules exhibited urate oxidase activity on schedule. In both sets of experiments (Fig. 1, C and D) the appearance of urate oxidase activity occurred at the expected time of emergence of the pupae which were the source of the donor tubules.

Since Malpighian tubules were first removed from donor pupae and then transplanted into the abdomen of a host with a fine glass needle, it was possible that these techniques reproducibly damaged the tubules and thus caused the delay in the expression of urate oxidase activity.

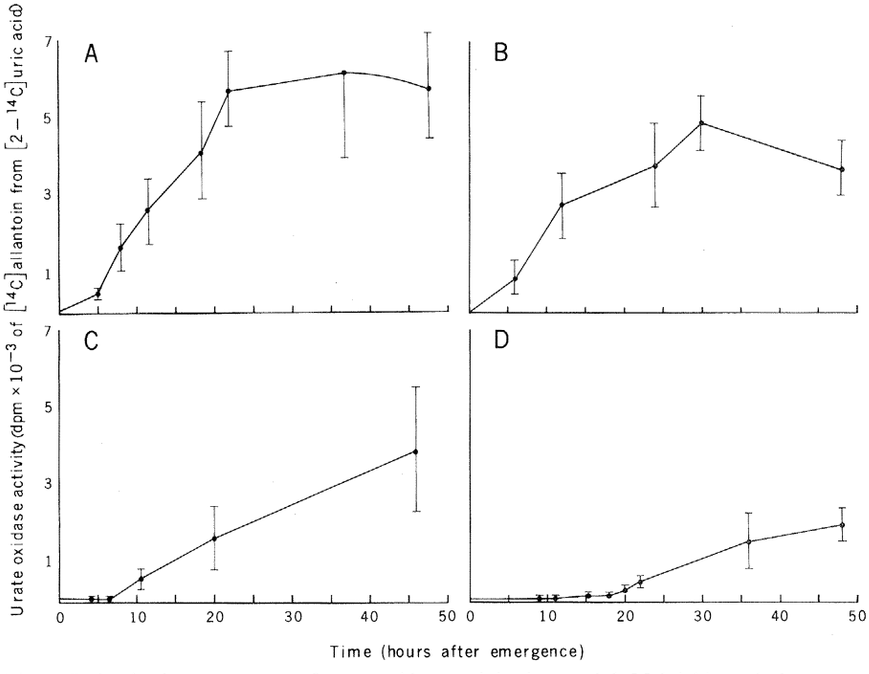

Fig. 1. Delay in the appearance of urate oxidase activity in preadult Malpighian tubules transplanted to newly emerged adults. Urate oxidase activity in single tubules was measured by a radiochemical assay (*19*). Urate oxidase activity was expressed as disintegrations per minute (dpm) of [^{14}C]allantoin formed from [2-^{14}C]urate per minute of reaction time (*23*). Counting efficiency was 79 percent. Error bars indicate standard deviations for each time point which is the mean of three to eight independent determinations. All donors and hosts were xanthine dehydrogenase–deficient females. (A) Urate oxidase activity increased rapidly without delay upon emergence of the adult. (B) Malpighian tubules were removed from newly emerged xanthine dehydrogenase–deficient adults and immediately transplanted into newly emerged hosts. Tubules were retrieved 6, 12, 24, 30, and 48 hours after transplantation, and assayed for urate oxidase activity. (C) Malpighian tubules from pupae with 10 ± 3 hours remaining until emergence were transplanted into hosts and assayed at the times indicated. (D) Malpighian tubules were removed from pupae with 24 ± 3 hours remaining until emergence, transplanted into newly emerged hosts, and then assayed at eight different times after transplantation. At least for the last 20 to 24 hours of the pupal period the delay in the appearance of urate oxidase activity was approximately equal to the calculated time remaining for development of the pupae which were the source of the donor tubules.

To test this hypothesis, tubules from newly emerged adults were transplanted into hosts which were also newly emerged from the puparium. Donor tubules from newly emerged flies behaved normally (Fig. 1B). No delay in the expression of urate oxidase in the donor tubules was observed (Fig. 1B). Thus, the act of dissection and transplantation did not inflict damage sufficient to cause a delay in the expression of urate oxidase in a transplanted tubule.

When a donor tubule began to exhibit urate oxidase activity after a delay of 20 hours (Fig. 1D) the rate of increase in activity was not as great as that observed in the host fly after emergence (Fig. 1A). Data (*12*) indicate that the regulation of the amount of urate oxidase activity in *Drosophila* is not an autonomous property of the Malpighian tubules. The level of urate oxidase activity in the tubules is regulated by the concentration of factors in the hemolymph. In comparison to a newly emerged adult, a 1-day-old adult that is deficient in xanthine dehydrogenase has considerably lower quantities of the hemolymph factors (*12*). Thus, after transplantation to a newly emerged adult, a donor tubule which then delays the expression of urate oxidase activity for 1 day would be expected to exhibit a reduced level of urate oxidase activity after the delay period.

We interpret our results as indicating the existence of a timing device in the pupal Malpighian tubules. This developmental clock appears to determine the time when the tubules will be competent to respond to the factors in the hemolymph which in turn regulate the amount of urate oxidase activity. The factors in the hemolymph which stimulate a high level of urate oxidase activity in the host tubule do not prematurely provoke the appearance of urate oxidase activity in donor tubules taken from pupae during the last 24 hours prior to emergence. At least for the last 24 hours of the pupal period the delay in the appearance of urate oxidase activity in donor tubules was approximately equal to the calculated time remaining for development of the pupae which were the source of the donor tubules. The donor and host tubules bathed in the same hemolymph show autonomous temporal profiles for the expression of urate oxidase activity (Fig. 1).

The timer in the tubules assures synchrony between the appearance of urate oxidase activity in the tubules and emergence of the adult from the puparium. Both the appearance of urate oxidase activity in the adult Malpighian tubules and emergence occur only once during the

life cycle of *Drosophila*. Emergence in *Drosophila*, a nonperiodic event, is controlled by a circadian clock (*21*) localized in the head region (*22*). It is possible that components of the same clock regulating emergence also function in the Malpighian tubules to control the time of appearance of urate oxidase activity in the tubules.

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23. A 5-μl sample of the reaction mixture was removed at 2 minutes and again at 6 minutes after initiation of the reaction with 2-[^{14}C]urate. The amount of [^{14}C]allantoin formed per minute of reaction time was calculated from the difference in disintegrations per minute between the two 5-μl samples.
24. We thank M. Wing for expert technical assistance, and E. Goudsmit, P. Ketchum, and P. Friedman for helpful discussions. This work was supported by funds from the Oakland University Research Committee, an Oakland University Alumni Association undergraduate research grant to D.H.J., and NIH Biomedical Science Support grant 5-07-RR07131.

2 February 1977

Compressional and Surface Waves in Sand: Used by Desert Scorpions to Locate Prey

Abstract. *Loose sand conducts compressional and surface (Rayleigh) waves at relatively low velocities (95 to 120 meters per second and 40 to 50 meters per second, respectively) compared to other natural substrates. For frequencies between 1 and 5 kilohertz, the specific attenuation factor,* Q, *for sand is 18. Compound slit sensilla on basitarsal leg segments of sand-dwelling scorpions respond to surface waves generated by movements of insects as far as 50 centimeters away, and tarsal sensory hairs respond to higher-frequency (mostly compressional-wave) components of the signal.*

The nocturnal sand scorpion *Paruroctonus mesaensis* captures prey with its pedipalps after an abrupt turn and forward movement toward small disturbances of the substrate nearby (*1*). For cricket-sized insects walking on the surface of the sand less than 20 cm away, the scorpion can determine both direction and distance of the prey's location with enough accuracy to consistently place its pedipalps within a few centimeters of the target in a single movement. Two or more movements are usually required to locate prey farther than 20 cm away. The range, accuracy, and speed of localization are such that in three or four orientation movements, lasting about 1 second each, the scorpion can position itself over a burrowing cockroach (*Arenivaga investigata*) that was initially 50 cm away. Thereafter, movements of the cockroach provoke a fast digging behavior from the scorpion that uncovers the prey. Fast and accurate localization of the cockroaches is essential for successful hunting, because these burrowers are also highly sensitive to disturbances of the substrate and respond to a scorpion's approach by burrowing deep into the sand.

Behavioral experiments with scorpions (*1*, *2*) have shown that they use information propagated through sand to locate their prey. Natural solids are not considered important avenues of information transfer, since they are generally heterogeneous and inelastic, or the conduction velocity and wavelength of the signals they conduct are too large to convey biologically useful information other than to warn of a disturbing force nearby (*3*). To determine the direction and distance of disturbances several decimeters away, however, the scorpion must sense small changes in some feature (for example, time of arrival, intensity, or frequency) of the signal that activates its spatially separated sense organs.

To identify the signals detected by the scorpion, I measured some of the physical properties of substrate vibrations in desert sand. The results show that, in this medium, biologically useful signals are transmitted as low-velocity surface (Rayleigh) waves (*4*, *5*) and compressional body (*P*) waves. Each type of wave is detected by a different sense organ on the legs of the scorpion, and input from one of these receptors is used to determine the direction of the wave source.

Standard techniques for three-dimensional seismic modeling were used to determine the types of waves propagated through sand and the extent to which sand acts as an elastic medium at biologically important frequencies. The model was a Styrofoam box (30 by 50 by 40 cm deep) filled with loosely packed desert sand. Substrate displacement pulses were generated with a piezoelectric crystal on the sand surface, and another piezoelectric transducer (*6*) was used as a receiver.

Two separate waves were distinguished in the seismogram recorded from sand (Fig. 1A) when the receiver was placed at the surface with its movement-sensitive axis oriented radially with respect to the vibration source. The first wave to arrive at the receiver had a short period (about 0.5 msec). It was followed by a slower surface wave of longer period (1.2 to 1.5 msec). The amplitude of the fast wave was diminished and the amplitude of the slow wave was increased when the movement-sensitive axis of the receiver was oriented perpendicular to the surface. When the receiver was oriented so as to be sensitive to transverse displacements, neither wave was recorded. If an absorbant object (for example, my hand or a rubber brick) was placed on the surface between the source and receiver, the slow wave diminished in amplitude or disappeared without change in the fast wave. The amplitude of the slow wave progressively diminished to zero as the receiver was placed at increasing depths (down to 3 cm) below the surface.