Saltatory Motility of Uninserted Trichocysts and Mitochondria in *Paramecium tetraurelia*

Abstract. *In the subcortical regions of the ciliate* Paramecium tetraurelia, *mitochondria and uninserted trichocysts each display saltatory motility with individual characteristics, making them distinguishable from each other and from cellular cyclosis. The saltatory motion of trichocysts is implicated as the means of transporting new trichocysts from the cytoplasm to their ultimate locations in the cellular cortex. Saltatory motion may also be a factor in the intracellular distribution of mitochondria.*

Saltatory motion is a phenomenon observed in eukaryotic cells of many different taxonomic groups (*1*). Among the protozoa, saltations have been studied mostly in rhizopods (*2*), although some brief observations have been reported in the ciliates (*3*). The existence of saltatory motion of mitochondria and of uninserted trichocysts in the subcortial cytoplasm of the ciliate *Paramecium tetraurelia* is reported here.

Paramecium tetraurelia, stock 51 sensitive, mating type O, was obtained from the Sonneborn stock collection, Indiana University. Cells were cultured by standard techniques (*4*), in baked lettuce medium in depression slides at 27°C, to give a growth rate of one fission per day. For microscopic observations of living cells, a few individuals were placed in a rotocompressor (Biological Institute, Philadelphia), which was adjusted to immobilize the cells with the minimum distortion possible. Observations were made with oil-immersion phase-contrast or Nomarski differential-phase optics. Attention was directed to the cytoplasmic region immediately beneath the array of structures known as the cellular cortex (*5, 6*). Approximately 1 to 2 μm into the cell, many mitochondria (*6, 7*) and uninserted trichocysts can be found. Uninserted trichocysts can be distinguished from inserted trichocysts since the former usually lie parallel to the plasma membrane and are observed side-on, under these conditions, while the latter are oriented perpendicular to the plasma membrane and are observed end-on.

The motility of trichocysts and mitochondria was monitored by real-time observations with a calibrated ocular reticle and a stopwatch, and by analysis of cinemicrographs taken at 24 frames per second with a Locam system (Redlake Corp., Santa Clara). Care was taken to ensure that the organelles observed were not in a region displaying cyclosis, in order to avoid confusing any organelle-specific motility with the bulk flow of cyclosis. Parameters of cyclotic flow were measured during periods of maximum cyclotic activity in other cells which were immobilized by the antiserum technique (*8*).

Mitochondria and uninserted trichocysts in the subcortial region of *P. tetraurelia* display discontinuous, individual, and independent movements which can be considered saltatory in nature (Figs. 1 and 2) (*1*). Table 1 summarizes the mean velocities and displacements and the saltatory indexes (*9*) of uninserted trichocysts and of mitochondria in the subcortical region of cytoplasm, and the mean velocity of cyclosis of antibody-immobilized stock 51 cells.

The individual and independent nature of motility of trichocysts and mitochondria is in strong contrast to the bulk flow of cyclosis. Although two organelles may be initially adjacent to each other in the subcortical cytoplasm, one may saltate many micrometers while the other remains motionless. This certainly does not occur in cyclosis, where all materials are swept along in a bulk flow and no individual motility is observed. Saltatory motility of the two organelles is also observed even when cyclosis is "shut down" in a cell (*8*). This qualitative difference between cyclosis and organelle saltatory motility is paralleled by the quantitative difference between the mean velocity of cyclosis and the mean velocities of saltation of the two organelles. The mean saltatory velocities and the saltatory indexes of trichocysts and mitochondria are also significantly different from each other. Qualitatively, the motility of one class of organelle does not appear to affect the motility of the other. This difference in quantitative parameters and qualitative appearance of

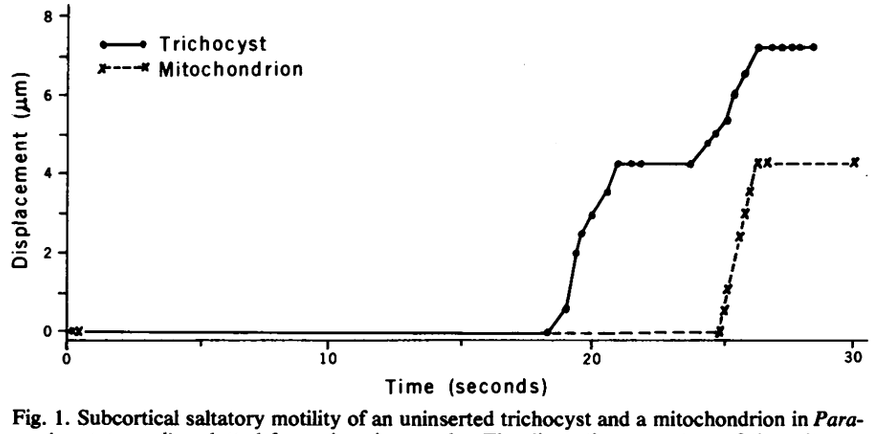

Fig. 1. Subcortical saltatory motility of an uninserted trichocyst and a mitochondrion in *Paramecium tetraurelia*, plotted from cinemicrographs. The discontinuous nature of the saltatory motility of the two organelles is clearly demonstrated. Displacement is measured from the initial locations. The trichocyst is observed to make two consecutive moves with a 3-second period of rest between the moves. The parameters of saltatory motion vary greatly for both organelles; this plot gives only single examples for each organelle and should not be construed as representing the means.

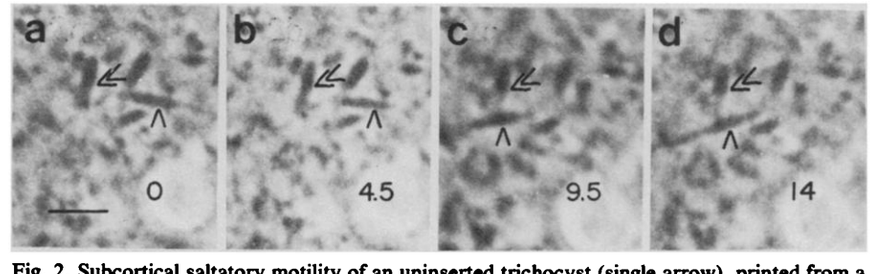

Fig. 2. Subcortical saltatory motility of an uninserted trichocyst (single arrow), printed from a survey film. A motionless trichocyst (double arrow) serves as a stationary reference point. The time of observation in seconds is marked on each frame. Between (b) and (c), the trichocyst was observed to saltate 5 μm in 5 seconds. At other times during observation, the trichocyst displayed only a weak Brownian motion. Magnification, 2300; scale bar, 3 μm.

Table 1. Parameters of intracellular motility in *P. tetraurelia*. Values are means ± standard errors. Abbreviation: N.A., not applicable to cyclosis.

Observation	Cells observed	Observations	Mean velocity during movement (μm/sec)	Mean displacement (μm)	Saltatory index
Saltatory motility					
Trichocysts	13	42	0.82 ± 0.08	3.0 ± 0.27	0.25 (32/127)
Mitochondria	8	29	1.2 ± 0.13	2.6 ± 0.41	0.37 (70/191)
Cellular cyclosis	12	142	2.6 ± 0.10	N.A.	N.A.

the two organelles could reflect independence in motility and could be based on different motility mechanisms or on different responses to a common force-generating mechanism.

The characteristics of saltatory motion of uninserted trichocysts are noteworthy. These movements are generally parallel to the cortex in this system, and individual saltations of 10 μm and more have been recorded. A trichocyst moves either tip first or body first—that is, with the long axis of the organelle parallel to the direction of movement (Fig. 2). A streamlined mode of movement is consistent: trichocysts have not been observed to saltate sideways. This characteristic of trichocyst saltatory motility is in marked contrast to cyclotic transport of trichocysts, in which the organelle is carried with random orientation of its long axis to the direction of cyclotic flow. If a change in direction is made between successive saltations, it is accomplished by a rotational reorientation of the trichocyst before the second jump. These activities give the observer the impression that the trichocyst is being "pulled" during its saltations.

The concept of saltatory motility of mitochondria and uninserted trichocysts has intriguing implications and gives added significance to the understanding of some of the cellular events of *P. tetraurelia*. Preliminary results (*10*) have indicated that two mutant cell lines which display a phenotype of mature, but uninserted, trichocysts (*11*) are incapable of transporting their trichocysts by saltatory motion. Observations of trichocyst replacement in the cortex (*12*) following massive trichocyst extrusion stimulated by electroshock (*13*) indicate that the motility shown by new trichocysts being transported to the cortex for insertion is saltatory. The parameters of trichocyst motility during cortical insertion (that is, velocity and displacement) are statistically indistinguishable from the trichocyst motility reported here (Table 1) in survey observations. Therefore, saltatory motion of trichocysts appears to be essential for their insertion into the cortex.

Perasso and Adoutte (*14*) found that the distribution of genetically marked mitochondria after transfer to an unmarked host cell is extremely rapid. Since many of the mitochondria in *P. tetraurelia* are located in the cortex (*6, 7*) and thus away from the cyclotic regions of the cell (*8*), it may be reasonable to suggest that mitochondrial saltatory motility could be a significant factor in the rapid intracellular mixing of mitochondria.

The observations described above suggest that subcortical saltatory motion may be involved in the distribution of uninserted trichocysts and of mitochondria in the noncyclotic, cortical regions of the cell. However, the apparent direct involvement of saltatory motion in transporting the uninserted trichocyst to the cortex for insertion and the genetic correlation between cortical insertion and saltatory motility of trichocysts, as mentioned above, suggest that saltatory motion is an important step in the functional development of the trichocyst. Saltation appears to be the type of motility displayed by the newly assembled trichocyst on its way from the site of assembly in the cytoplasm (*13*) to the cell surface for insertion. This would implicate saltatory motion in the development and maintenance of the normal cellular phenotype of *P. tetraurelia*: the insertion of approximately 4000 trichocysts in the cortex. In addition to paramecia, many other cell systems exhibit the use of saltatory motility in the intracellular transport and localization of various inclusions, such as the cortical localization of echinochrome granules in sea urchin eggs following fertilization, and the axoplasmic transport of materials such as neurotransmitters essential for the proper function of the neuron (*1, 15*). Saltatory motility would thus appear to be an important element in the process of intracellular morphogenesis in eukaryotic cells.

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9. The saltatory index is defined as the number of particles observed to saltate in 1 minute divided by the total number of particles of the same type, potentially available for saltation in a particular region [R. Roisen, quoted in (*1*)].
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Gonadotropin-Releasing Hormone in Milk

Abstract. *The hypothalamic hormone gonadotropin-releasing hormone (GnRH) has been found in milk of man, cow, and rat. Radioimmunoassays of acidified milk indicate concentrations of GnRH ranging between 0.1 and 3 nanograms per milliliter. Multistep extractions, followed by electrophoresis, reveal gonadotropin-releasing activity in the fraction that comigrates with the GnRH-marker. A second hypothalamic hormone, thyrotropin-releasing hormone, is present in milk at a much lower concentration. "Milk-GnRH" may influence the secretion of the gonadotropic hormones in neonates.*

The gonadotropin-releasing hormone (GnRH), which activates gonadal function by promoting gonadotropin secretion from the anterior pituitary, is present in minute amounts in the hypothalamus [approximately 100 ng and 5 ng in human and rat, respectively (*1*)]. Measurement of the peptide in the peripheral circulation has presented many difficulties (*2*) and only elaborate extrica-