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Video Essay

Self-Centering in Cytoplasmic Fragments of Melanophores[1]V

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INTRODUCTION

The radial array of cytoplasmic microtubules (MTs) provides routes for intracellular transport and defines spatial organization of cytoplasm through interaction with molecular motors bound to membrane organelles (Kellogg *et al.*, 1994; Hirokawa, 1998). The array is believed to be organized by the centrosome, which is capable of nucleating MTs. Our recent studies of pigment transport (Rodionov and Borisy, 1997a) (reviewed in Haimo, 1997) and MT dynamics (Rodionov and Borisy, 1997b) in cytoplasmic fragments of melanophores demonstrated that nucleation by the centrosome is not an exclusive pathway for organizing microtubules. We demonstrated that a radial array of cytoplasmic MTs can form by self-organization and, moreover, that a mechanism exists that maintains the focus of the array at the cell centroid.

Fish melanophores are pigment cells whose only function is aggregation of pigment granules at the center or redispersion throughout the cytoplasm. The granules move along radial microtubules (MTs) by means of molecular motors of dynein (aggregation) or kinesin (dispersion) families (Schliwa, 1984; Obika, 1986; Haimo and Thaler, 1994). Microsurgically produced cytoplasmic fragments of melanophores organize a radial array of MTs with correct polarity orientation (plus ends at the periphery) and aggregate pigment at its center (Matthews, 1931; McNiven *et al.*, 1984; McNiven and Porter, 1986, 1988).

VIDEO SEQUENCES

Video Sequence 1: Aggregation of Pigment Granules in a Melanophore Fragment

A cytoplasmic fragment of a melanophore was dissected with a glass microneedle and induced to aggregate pigment granules 60 min after dissection. Irregular motion of the pigment granules resulted in accumulation at the fragment center (Figure 1).

Aggregation to the center required MT dynamics and apparently did not depend on the centrosome components γ-tubulin (Oakley, 1994) and pericentrin (Doxsey *et al.*, 1994). Randomly arranged MTs transformed into a radial array when pigment was induced to aggregate at the center, but the MT distribution returned to random arrangement during the course of dispersion. Formation of the radial MT array depended on the interaction of MTs with pigment granules and required activity of a minus end–directed motor, cytoplasmic dynein. We suggested that the motion of pigment granules along MTs organized MTs into a radial array. A possible mechanism involved interaction of each granule with more than one MT and required that not only the granules moved toward the MT minus ends but MTs themselves were transported with plus ends leading by the motors bound to the granules so that minus ends gradually came together.

The radial array persisted in the aggregated state as long as the minus end dynein motors on the pigment granules were active. Live observation of behavior of fluorescently labeled MTs in the fragments with aggregated pigment showed that new MTs continuously emerged from the pigment mass or released from the aggregate and depolymerized. Such a mechanism of subunit exchange suggested that the pigment aggregate was capable of MT nucleation. MTs of incorrect polarity orientation that occasionally self-nucleated in the cytoplasm were eliminated by transport across the pigment mass. Thus, the aggregate of pigment granules maintained a radial arrangement of MTs by nucleating and transporting the MTs.

Video Sequence 2: Behavior of MTs in a Fragment with Aggregated Pigment

A fragment was dissected from a melanophore injected with fluorescently tagged tubulin subunits, and sequential images of labeled MTs and of pigment granules in the fragment were obtained with a cooled charge-coupled device camera. MTs emerged continuously from the pigment aggregate located at the cen-

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V Online version of this essay contains video information for Figures 1–4. Online version available at www.molbiolcell.org.

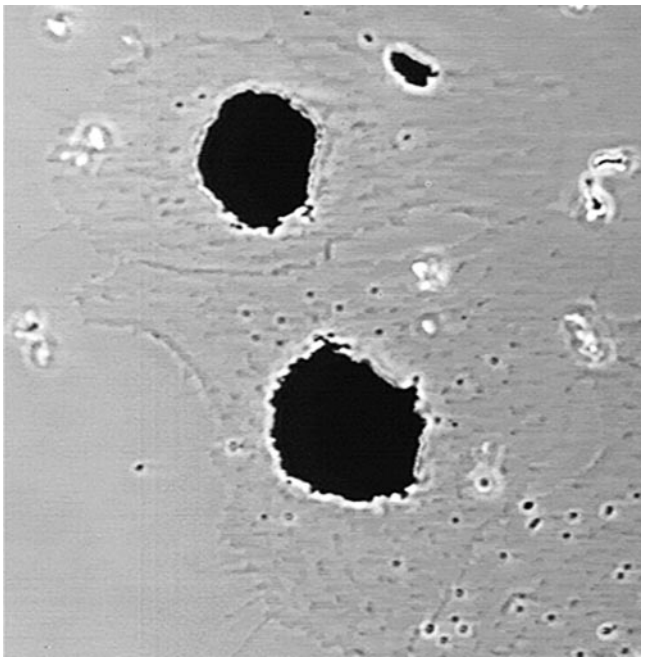

Figure 1. Video sequence 1 shows aggregation of pigment granules in a melanophore fragment.

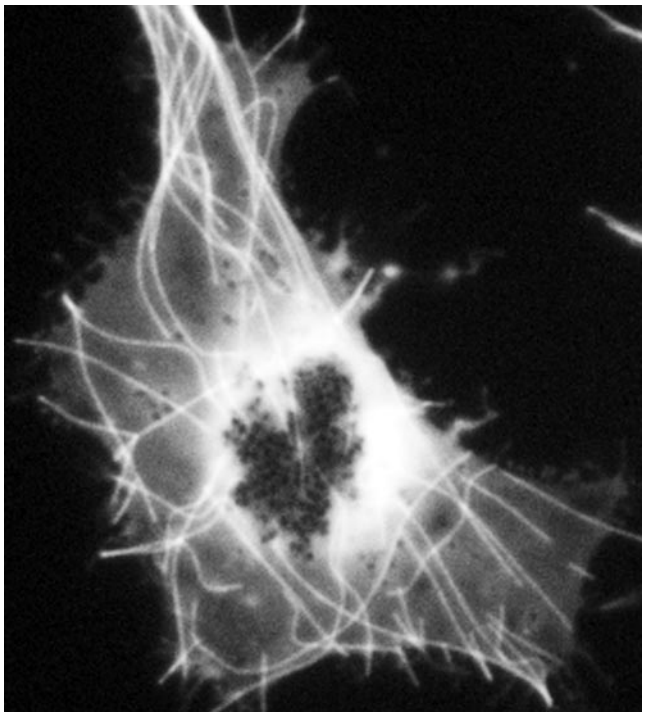

Figure 2. Video sequence 2 shows behavior of MTs in a fragment with aggregated pigment.

ter of the fragment and grew persistently to the cell periphery by addition of subunits at their distal ends or released from the aggregate and depolymerized at their proximal ends (Figure 2). Short MTs moved to the periphery by treadmilling, polymerization at one end and depolymerizatioin at the other (top right part of the fragment). MT self-nucleated in the cytoplasm (bottom right part of the fragment) was eliminated by transport by motors attached to the granules.

The location of a focal point of radial MTs labeled with the pigment aggregate matched the center of a fragment. The most clear demonstration of existence of a self-centering mechanism was provided by nascent fragments.

Video Sequence 3: Redistribution of the Pigment Aggregate to the Center in a Nascent Fragment

The fragment was dissected with a glass microneedle and immediately induced to aggregate pigment granules. The pigment aggregate initially formed at the proximal (cut) edge of a fragment and then relocated to the center with kinetics approximated by a single declining exponential curve; $t_{1/2} = 150 \pm 55$ s (Figure 3).

Self-centering of pigment could result from an attraction to the fragment center or from avoidance of cell periphery. Because the centrosome equivalent was undetectable in the fragments, we considered that the centering mechanism involved an interaction of MTs with the fragment surface. We attempted to test this relationship by varying the geometry of the fragment and determining whether the aggregation pattern was specified accordingly. Because the general pattern of

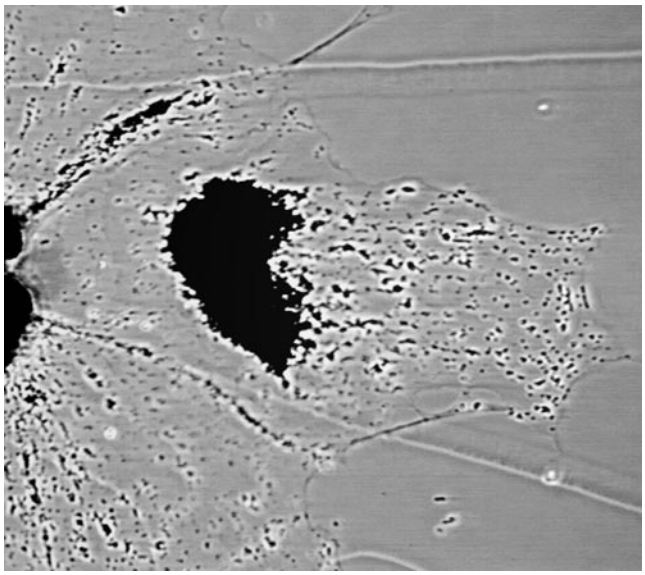

Figure 3. Video sequence 3 shows redistribution of the pigment aggregate to the center in a nascent fragment.

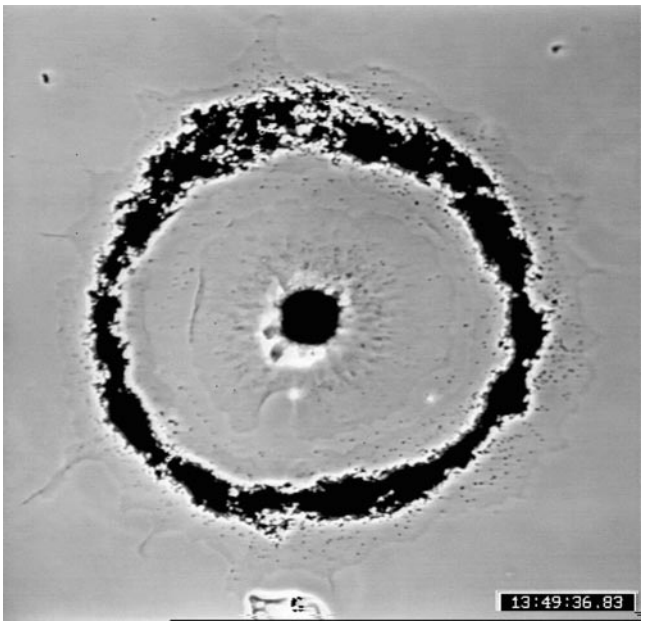

Figure 4. Video sequence 4 shows pigment aggregation in the toroid-shaped fragment.

pigment aggregation was to the centroid of the fragment, we devised a shape, namely a torus in which this pattern was impossible because the centroid lay outside the fragment.

Video Sequence 4: Pigment Aggregation in the Toroid-shaped Fragment

A melanophore was dissected with a microneedle producing a toroidal fragment and a discoidal remnant containing the centrosome. After stimulation with adrenalin, pigment granules in a toroidal fragment moved to a zone equidistant from both the outer (preexisting) and inner (newly formed) edges or the fragment, whereas pigment in the discoidal remnant of the parental cell aggregated to the centroid as normal (Figure 4).

Thus, consistent with our prediction, granules in the toroid moved away from the margins even though the direction for approximately half of them was opposite to that for pigment in intact cell or remnant discoid. We suggest that the information for locating the center is derived by interaction of MTs with the cortex. The driving force for centering is most likely explained by addition of the new subunits to MTs at their minus ends at the aggregate (Rodionov and Borisy, 1997a), although contribution of plus end growth at the cortex cannot be completely excluded.

An emerging body of evidence suggests that formation of MT arrays by self-organization mechanisms that require activity of molecular motors is a general phenomenon (Hyman and Karsenti, 1996; Merdes and Cleveland, 1997). Self-centering activity found in melanophore fragments provides a dramatic illustration of this idea. Further experiments will help evaluate relative roles of self-organization and nucleation at the centrosome in formation of MT arrays in living cells.

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