Cytochalasin B: Effects on Microfilaments and Movement of Melanin Granules within Melanocytes

Abstract. *The intracellular translocation of melanin granules within both epidermal and dermal melanocytes of* Rana pipiens *is influenced by cytochalasin B. Cytochalasin B prevents dispersion of pigment granules by melanocyte-stimulating hormone (MSH) and causes centripetal movement of pigment granules that have been dispersed by MSH. Microfilaments are abundant in the dendritic processes of epidermal melanocytes in which pigment granules have been dispersed by MSH. Microfilaments are dramatically reduced in number in the processes of melanocytes that have been lightened by cytochalasin B. These observations suggest that microfilaments mediate dispersion of pigment granules.*

Rapid alteration of skin color in fish, amphibians, and many reptiles is associated with the intracellular rearrangement of melanin granules within dermal and epidermal melanocytes. When pigment granules are dispersed, the skin is dark; when the granules are aggregated, the skin is light (*1*).

Many compounds influence the intramelanocytic distribution of melanin granules in the skin of *Rana pipiens*. Melanocyte-stimulating hormone (MSH) and β-adrenergic agents cause dispersion of pigment granules. Melatonin and α-adrenergic agents cause aggregation of pigment granules (*2*).

There is convincing evidence that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) mediates pigment granule dispersion (*3, 4*). The amount of cyclic AMP in frog skin is increased after exposure to MSH (*5*), and granule dispersion is effected by cyclic AMP (*3*) as well as by caffeine, an inhibitor of adenosine 3′,5′-monophosphate phosphodiesterase. Conversely, the amount of cyclic AMP in frog skin is reduced by norepinephrine and melatonin, compounds that cause granule aggregation (skin lightening) (*6*). We describe experiments designed to identify the transducer between cyclic AMP and dispersion of pigment granules.

Cytochalasin B, a compound present in the filtrates from cultures of several different fungi (*7*), causes breakdown of microfilaments (*8*). When the influence of this compound on translocation of pigment granules was examined, we found that cytochalasin B (i) prevented the dispersion of pigment granules by MSH and (ii) caused the aggregation of pigment granules in dermal and epidermal melanocytes that had been treated with MSH.

When frog skin was exposed to cytochalasin B (10 μg/ml) for 90 minutes and then exposed to α-MSH (1 to 20 unit/ml) without removal of cytochalasin B, the pigment granules in the epidermal and dermal melanocytes remained aggregated. When cytochalasin B was removed by washing the skin with Ringer solution for 4 hours, both epidermal and dermal melanocytes were darkened by α-MSH (1 unit/ml). If the skin was first darkened by α-MSH (1.5 unit/ml), the addition of cytochalasin B (20 μg/ml) caused aggregation of pigment granules in both dermal and epidermal melanocytes (Fig. 1).

Prevention of the centrifugal trans-

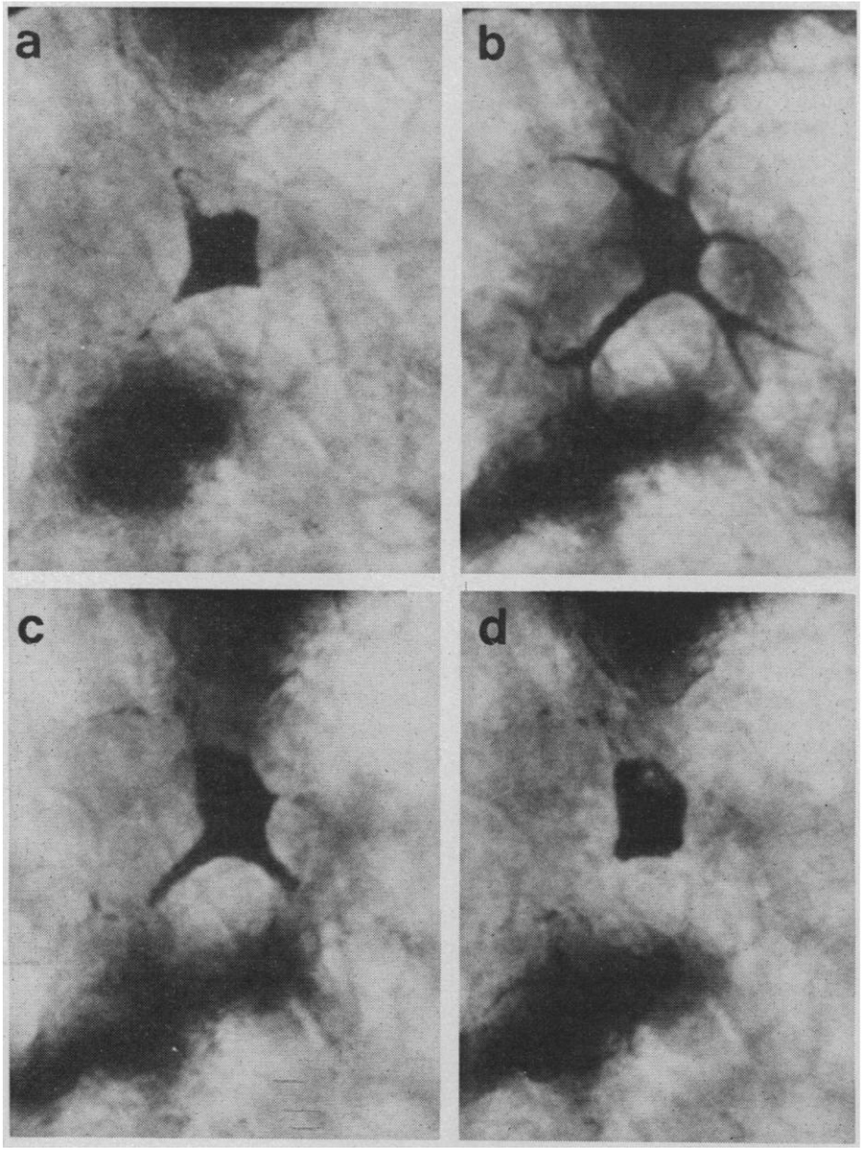

Fig. 1. Effect of cytochalasin B on epidermal melanocytes darkened by MSH. (a) An epidermal melanocyte is seen with the pigment granules aggregated in the perikaryon. (b) The same epidermal melanocyte is seen 90 minutes after exposure to α-MSH (1.5 unit/ml). (c) Pigment granules are partially aggregated 13 minutes after the addition of cytochalasin B (20 μg/ml) to the solution containing MSH. (d) Melanin granules are almost completely reaggregated 100 minutes after the addition of cytochalasin B. A small translucent area has appeared near the top of the cell body; this is caused by the melanocyte nucleus, which has displaced pigment granules. Pigment granules are seen trapped in the cell processes at the upper left and lower left ($\times$ 790).

location of melanin granules by cytochalasin B suggested that microfilaments are involved in the translocation. Ultrastructural examination supported this notion.

Electron micrographs of epidermal melanocytes from skins darkened by MSH showed occasional microtubules and abundant microfilaments (Fig. 2), which filled the central area of the dendritic processes. Individual microfilaments were 65 to 75 Å in diameter, and some coursed unbroken for more than 1 μm. Skins that had been darkened with MSH and then lightened with cytochalasin B sometimes revealed a sharp delineation (Fig. 3a) between the proximal portion of the melanocyte process, which contained pigment granules, and the distal portion, which contained endoplasmic reticulum, numerous ribosome-sized particles, mitochondria, microtubules, and rare microfilaments (Fig. 3b). Melanin granules were sometimes sequestered in the cell process after exposure to MSH and cytochalasin B. In this situation, the granules were still associated with microfilaments.

Heretofore, microfilaments have been implicated in alterations of cell morphology and motility (*8, 9*). Our studies indicate that microfilaments can participate in the intracellular translocation of specific organelles (melanin granules). The location of mitochondria appears uninfluenced by the action of cytochalasin B.

These observations suggest that microfilaments occupy an intermediary position between MSH-dependent activation of adenylate cyclase and dispersion of melanin granules in the amphibian melanocyte. Our experiments do not identify the basis of the chemical coupling between cyclic AMP and microfilaments or of the mechanical coupling between microfilaments and granules.

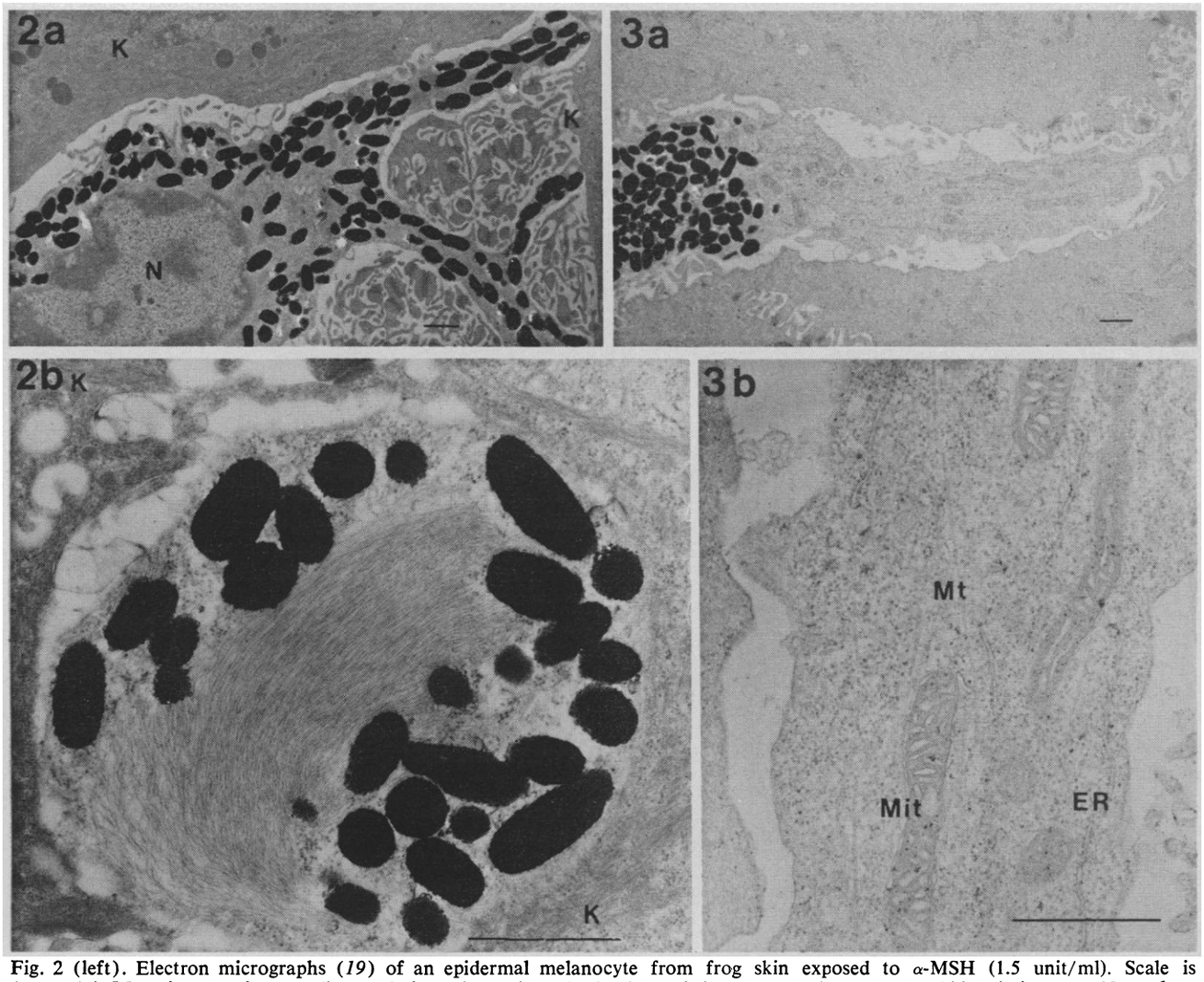

Fig. 2 (left). Electron micrographs (*19*) of an epidermal melanocyte from frog skin exposed to α-MSH (1.5 unit/ml). Scale is 1 μm. (a) Melanin granules are dispersed throughout the arborizations of the melanocytic processes. Abbreviations are *N*, nucleus of melanocyte and *K*, keratinocyte ($\times$ 4,750). (b) An epidermal melanocyte process is located between keratinocytes (*K*). Melanin granules are located peripherally near the plasma membrane, and the center of the process is filled with microfilaments coursing in the long axis of the process. The cell process has been sectioned obliquely ($\times$ 23,400). Fig. 3 (right). Electron micrographs (*19*) of an epidermal melanocyte process in skin that had been exposed to α-MSH (1.5 unit/ml) for 2 hours and then to cytochalasin B (4 μg/ml) and α-MSH (1.5 unit/ml) for 2 hours. Scale is 1 μm. (a) An abrupt line of demarcation is seen between the distal portion of a melanocyte process, which contains mitochondria, and the proximal portion, which is filled with melanin granules ($\times$ 5,000). (b) A segment from the same melanocyte process is shown. The cytoplasm is filled with ribosome-sized particles. Mitochondria (*Mit*), microtubules (*Mt*), and elements of endoplasmic reticulum (*ER*) are oriented parallel to the long axis of the process. Microfilaments are absent ($\times$ 22,800).

If microfilaments function in dispersal of pigment granules, it is obligatory to reexamine the function of microtubules in translocation of pigment granules. Marsland's observation (*10*) that pigment granules were dispersed by high hydrostatic pressure suggested that a gel-to-sol transition occurred in darkening. Malawista (*11*) found that prior treatment of frog skin with colchicine enhanced the darkening effect of MSH and inhibited the lightening that follows removal of MSH; he proposed that colchicine affected cytoplasmic viscosity in frog melanocytes. Colchicine also disrupts microtubules (*12*) and decreases the rate of pigment aggregation in melanocytes in *Fundulus heteroclitus* scales in response to epinephrine (*13*). The darkening action of colchicine can be attributed either to interference with microtubular structure or with the sol-to-gel equilibrium.

Studies of *F. heteroclitus* melanocytes with the electron microscope (*14, 15*) indicate that pigment granules move through channels surrounded by microtubules. Because microtubules are arranged parallel to the long axis of the melanocytic process, it has been suggested that microtubules function as a cytoskeleton or perhaps provide tracks for the granules (*13, 14*). Wise (*16*) did not find such an orderly arrangement of microtubules in his electron microscopic examination of melanocytes of *Xenopus laevis* and *Hyla regilla.*

If colchicine inhibits pigment granule aggregation by disrupting microtubules and cytochalasin B inhibits pigment granule dispersion by disrupting microfilaments, then the following mechanism for pigment granule translocation can be tendered. Dispersion of pigment granules is effected by microfilaments; when microfilaments are destroyed, pigment granules move centripetally (*17*). Intact microtubules are required for pigment granule aggregation but not for dispersion. This model is supported by the inhibition by vinblastine of the lightening effect of cytochalasin on frog skin darkened with melanocyte-stimulating hormone (*18*).

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17. These observations are supported by studies by S. Malawista who found that cytochalasin B inhibits the decrease in reflectance (darkening) of frog skins caused by MSH (*Nature*, in press).
18. J. McGuire, unpublished observations.
19. Frog skins were fixed for electron microscopy at 30°C with 3 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer, *p*H 7.2, containing 0.1 percent $CaCl_2$. Skins were refixed in osmium tetroxide and embedded in epoxy resin by the method of A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969). Thin sections were counterstained with uranyl acetate and lead citrate.
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Tetraploid Origin of the Karyotype of Catostomid Fishes

Abstract. *Catostomid fishes appear to have* $2n(\rightarrow 4n?) \simeq 100$ *chromosomes. The Cyprinidae, from which catostomids probably diverged before the Eocene, usually have* $2n = 48$ *or 50 chromosomes. Preliminary cytophotometric measurements indicate an approximate doubling of DNA content of cells among catostomids.*

The fish family Catostomidae (suckers) comprises 12 genera and about 60 species of fishes confined to freshwaters of North America and eastern Asia. The jaw mechanism and other skeletal features indicate that the group evolved from an ancestor similar to the minnows, family Cyprinidae (*1*). The evolutionary divergence might have occurred in Asia (*2*) where cyprinids are especially diverse. Asian fossils of cyprinids from the Paleocene and catostomids from the Eocene (*3*) indicate separate evolutionary histories spanning at least 50 million years. All but two genera and species of catostomids are endemic to North America, where their fossil record also extends back to the Eocene. Cyprinids are unknown in North American fossil deposits earlier than the Oligocene, but the family is represented in North America by about 230 Recent species (*4*).

The chromosome number of cyprinids

Table 1. Diploid chromosome counts in catostomid fishes. Numerous cells with more than 85 chromosomes have been examined from each species. The tabulation of relatively countable spreads includes variability due to overlap and breakage of chromosomes. Columns indicate the number of fish in which dividing cells were examined and the frequency distribution of counts.

Taxon and locality	Fish (*N*)	Diploid cells (*N*)			
		<96	96–98	99–100	101–102
Ictiobus sp., Boone Co., Mo.	4	3	13	21	
Carpiodes carpio, Douglas Co., Kan.	3	1	12	19	
Cycleptus elongatus, Boone Co., Mo.	1		1	1	
Minytrema melanops, Monroe Co., Mich.	1	4			
Erimyzon sucetta, Van Buren Co., Mich.	3	2	2	4	
Hypentelium nigricans, Washtenaw Co., Mich.	6	2	8	9	3
Moxostoma duquesnei, Washtenaw Co., Mich.	2	3	2	1	
M. erythrurum, Washtenaw Co., Mich.	2	4	9	9	
M. macrolepidotum, Washtenaw Co., Mich.	2		2	2	
Catostomus commersoni, Washtenaw Co., Mich.	2	1	9		
C. latipinnis, Grand Co., Utah	3		2	4	
C. discobolus, Grand Co., Utah; Moffat Co., Colo.	3	1	4	4	
C. clarki, Coconino Co., Ariz.	3	2	4	1	
Hybrid *C. clarki* × *C. platyrhynchus*, Washington Co., Utah	2	1	4	3	