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CELLULAR ORGANELLE TRANSPORT AND POSITIONING BY PLASMA STREAMING

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Abstract: Our analysis of known data reveals that translocations of passively movable cellular organelles from tiny granules up to large cell nuclei can be ascribed to transport by streaming cytoplasm. The various behaviours, such as velocity changes during more or less interrupted movements, forth and back shuttling and particle rotation result from different types of plasma circulation. Fast movements over long distances, as observed in the large characean internodial cells occur in strong streams generated by myosin in bundles of actin filaments in the direction of the barbed filament ends. Slow movements with frequent reversions of the direction are typical for neuronal axons, in which an anterograde plasma flow, produced in a thin layer of membrane-attached actin filaments, is compensated by a retrograde stream, produced by dynein activity in the central bundle of microtubules. Here particle rotation is due to steep flow velocity gradients, and frequent changes of particle movements result from minor particle displacements in radial directions. Similar shuttling of pigment granules in the lobes of epidermal chromatophores results from the same mechanism, whereby the centrifugal movement along astral microtubules is due to flow generated by excess of kinesin activity and the centripetal movement to the plasma recycling through the intermicrotubular space. If the streaming pattern is reversed by switching to excess dynein activity, the moving granules are trapped in the high microtubule density at the aster center. The presence of larger bodies in asters disturbs the regular, kinesin-dependent microtubule distribution in such a way that a superimposed centrifugal plasma flow develops in the microtubule-dense layer along them, which is recycled in the microtubule-free space, created by their presence. Consequently, at excess kinesin activity, nuclei, mitochondria as well as chromosome fragments move towards the aster center until they reach a dynamically stabilized position that depends on the local microtubule density. These various behaviours are not rationally explainable by models based on a mechanical stepping along microtubules or actin filaments.

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INTRODUCTION

Translocation of organelles and other cell particles is a general phenomenon observed in living cells. Some movements serve vital aims such as the segregation of daughter chromosomes during mitosis, while others, including the circulation of small granules in cytoplasmic layers and strands of highly vacuolated plant cells, occur without any recognizable need. For about a century the phenomena have been ascribed to transport by streaming cytoplasm. This conclusion was drawn intuitively from careful observation on the course of movements and the simple fact that any movable object can be translocated by fluid currents. Such a view was later abandoned in favour of a mechanical model when it became clear that either myosin, kinesin, or dynein are essential for the various behaviours. It was now proposed that particles become attached to motor proteins, which then move them in a step-wise manner along the actin filaments or microtubules respectively [1]. It must be emphasized, however that many movements occur in the absence of suitably directed skeletal structures [2] and therefore cannot be explained by such a mechanism. Moreover, conclusive evidence for a corresponding motor-particle interaction, as required for the propulsion process, has not been obtained so far. While streaming cytoplasm is a most natural vehicle for intracellular particle displacement, the problem how the suitable flow patterns required in order to explain the complicated types of particle movements can be produced, has remained unsolved. We have previously shown that activities of various motor proteins are associated with the generation of plasma currents along microtubules and actin filaments [3]. Here we investigate how such currents can become merged into superimposed flow patterns by suitable arrangement of the skeletal elements, and show that a great range of particle behaviours can be ascribed to transport by such flow patterns.

PARTICLE TRANSPORT BY PLASMA STREAMING

Some general considerations

Streaming cytoplasm is a most natural carrier for particle translocation in living cells. The clearest example for this phenomenon is the transport of organelles along actin filament bundles in the cytoplasmic layer of the very large internodial cells of Characeae [4,5]. The particles are moved continuously over long distances whereby they reach velocities of 50 μm/sec, while plasma streaming velocities of up to 60 μm/sec have been measured [6]. In the closed space of the cell the displaced cytoplasm and enclosed particles must be recycled to the opposite ends of the filament bundles, which proceeds at low velocity and therefore less spectacularly, through the surrounding space. This recycling is the reason for the continuity of the observed flow. Notice that displacement by a

presently fashionable step mechanism would soon end up by an accumulation of all particles on one bundle end.

In contrast to the smooth, high-speed movement in the characean cells, particle displacement along individual actin filaments, as well as microtubules, proceeds in vitro with an average velocity of 0.5 μm/ sec and with frequent interruptions. The simple reason for this difference is that plasma currents generated by the respective motor proteins along single skeletal elements are weak and limited to a narrow layer [3]. Thus, particles can only immerse partially and for short periods into such currents. The strong plasma stream in characean cells is reached by merging of the numerous weak currents, which results from the bundling of the uniformly oriented actin filaments. It is obvious that the streaming required for the various cellular particle behaviours depends on the order of the skeletal elements which, of course, is itself affected by the streaming.

In the usually much smaller animal cells plasma streaming cannot be made observable directly and must therefore be deduced from the organization of the skeletal elements and the types of the motor activities involved. Membrane-attached actin filament bundles are well recognizable in filopodia that emerge from the rims of lamellipodia and neuronal growth cones. The filaments are maximally stretched because of the traction force exerted onto them by myosin motor activities [3]. Their orientation is more or less vertical to the plane of the cell surface [7], in agreement with the predictable plasma circulation, i.e. a fast flow towards the attachment sites inside and along the bundles and a slow opposite flow in the surrounding space. The distal bundle ends appear tapered owing the the centripetal flow component of the local plasma circulation. A similar shape of mitotic traction fibers, which consist of microtubules inserted into chromosomal kinetochores [8,9], can be ascribed to a same type of plasma circulation generated by kinesin motors. In contrast, when microtubules are associated with the organizing center by their minus-ends, as is the case in mitotic asters [10], the distal microtubule domains become maximally spread by the same motor activity. In the following sections we show how flow patterns required for various particle behaviours can be predicted from the spatial organization of the skeletal elements involved.

Axonal transport

Particle movements in neuronal axons are most suitable for studies on transport, because the translocations are essentially one-dimensional and can easily be followed in the thin axons that grow out of in vitro cultured neuronal cells. They contain a cortical layer of membrane-attached actin filaments with their free ends directed towards the cell body. This means that myosin motors generate an anterograde streaming that is recycled through the axon center. The flow is strengthened by a central bundle of microtubules, the plus-ends of which are predominantly oriented into the distal direction [11].

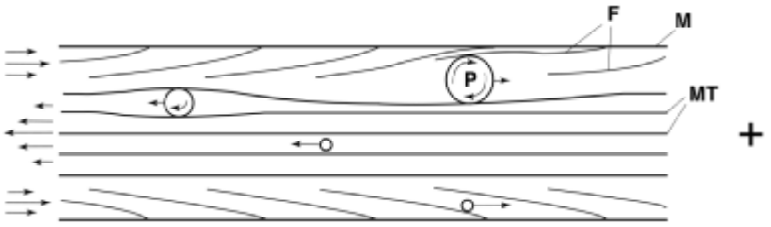

Fig. 1. Axonal transport by plasma currents. Diagram of an axonal length-section showing the central bundle of microtubules (MT) and the cortical, membrane (M)-anchored, actin filaments (F). The general distribution of velocities and directions of the plasma flow throughout the tube is indicated by lengths and directions of arrows on the left. Arrows attached to particles (P) indicate the velocity and direction of their translocation. Particle rotation is marked by arrows inside. Polarity of the microtubules is with the plus-ends (+) distal. For explanation see text.

A strong expression of motors exhibiting retrograde, microtubule-based motor activity, has been found in young neurons [12].This leads to a streaming pattern in which the retrograde flow is maximal in the axon center, decreases in radial direction, then changes into the anterograde direction, becomes maximal again at some distance from the membrane at which it finally becomes zero. It is obvious that the velocity of the opposite plasma currents in the narrow axons is strongly reduced by friction. Fig. 1 shows that all observed particle behaviours are characteristic for transportation by the described plasma circulation:

1) Particle velocities change continuously between zero and a maximum that depends on the particle size, usually around 2μm/sec [13]. The simple reason for this is that the actual velocity of a particle must match the average flow rate expected in the space occupied by it. Therefore it moves with maximal velocity when it is focused precisely to the center of a current but becomes slower when it deviates from it and stops when it enters the neutral zone between opposite currents. For a similar reason small particles that can be focused more accurately into a narrow current, reach higher velocities than the bulky ones that are bound to extend more deeply into the slower moving surroundings.
2) The direction of movement of individual particles alternates in a random manner [14]. This must be ascribed to transverse displacements from one current into the opposite one owing to Brownian motion or/and local streaming disturbances. As the displacement implies the passage through a more or less dense screen of actin filaments and microtubules, it occurs more frequently with small particles than with bulky ones.
3) Particles rotate during movement along axons as well as in other transport phenomena [2]. The spinning is a natural consequence of the exposure of

opposite particle surfaces to different flow rates and/or flow directions by asymmetric positioning in currents.

Particle movements measured over short time intervals can reach velocities of up to 5 μm/sec [13]. Obviously, because of the constantly changing velocities and alternating directions, average progression into one direction must be much slower when measured over longer periods of time. Accordingly, displacements of 0.25 mm per day have been observed for microtubules and neurofilaments in various animal axons in life [15,16]. This is only approximately 2% of the 7 μm/min velocity at which small arrays of microtubules can move into the growth cones of in vitro cultured neurons [17]. Notice that net transport for most axonal structures is required only during axon outgrowth. After maturation only structures produced in the cell body and consumed at the synapses, such as neurotransmitter-containing vesicles, remain subject to permanent net transport.

Organelle positioning and redistribution

Most important for plasma circulation involved in the continuous particle shuttling in axons, is a nearly parallel orientation of the microtubules and actin filaments along which the currents are generated. In asters composed of microtubules the streaming patterns must clearly differ. Intriguing patterns of particle translocation have been found in the distribution of pigment granules in dermal chromatophores of some classes of animals. These cells contain asters composed of microtubules that emerge from a dense core in the nuclear proximity, from where they radiate into more or less extended lobes of the cell surface. From mitotic cells it is known that the polarity of astral microtubules is with the plus-ends distal [10]. Pigment granules of 0.1 to 0.2 μm in diameter move along the microtubules and become either dispersed throughout the lobes or aggregated at the core of high microtubule density. Transition from one state to the other is hormonally regulated and takes place within one to two minutes.

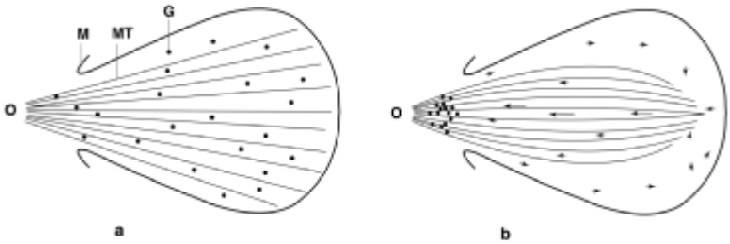

Fig. 2. Streaming-induced redistribution of pigment granules in the lobes of dermal chromatophores. a) Uniform distribution of granules (G) and microtubules at excess plus-end-directed motor activity. b) Granule aggregation and bundling of the distal microtubule domains caused by the plasma flow that is generated by the predominance of minus-end-directed motor activity. (O) Organization center of the aster-like arranged microtubules (plus-ends distal). Other symbols as in Fig. 1. For explanation see text.

Pigment dispersion has been shown to require plus-end-directed activity of kinesin [18]. The plus-end-directed motors generate outwards directed currents along the microtubules that lead to an optimal spreading of the distal microtubule domains and a plasma recycling to the aster center in the intermicrotubular space [Fig. 2a]. This must lead to a forth- and back-shuttling of the suspended particles in radial direction, comparable to the shuttling in neuronal axons. Such shuttling of pigment granules has been observed and described as saltatory movements [19]. Its natural result is a random distribution of pigment granules over the entire chromatophore lobe, in agreement with general observations. A similar behaviour of very small vesicular material is found in mitotic asters [20].

Pigment aggregation is known to depend on the activity of minus-end-directed dynein motors and is accompanied by a bundling of the distal microtubule domains [21,22]. Interestingly, in vitro systems reconstructed from mitotic asters of clam oocytes and lysates obtained from Fundulus heteroclitus melanophores at the two alternative stages exhibit the same different microtubule distributions [23]. The change of the microtubule distribution results from the reversed plasma currents, which lack the property to spread the distal microtubule domains. This allows the spontaneous occurrence of locally increased microtubule densities in which the currents along individual microtubules become superimposed by a plasma circulation as described above. The streaming direction inside such a bundle is towards the aster center. The cyclic streaming pattern drives the distal microtubule domains towards each other [Fig. 2b] and causes the bundles to grow and fuse until one or a few very large ones remain per lobe. Pigment granules carried by the changed plasma flow now move swiftly towards the cell body where they are trapped in the narrowing intermicrotubular space at the aster center. In this way the lobes are efficiently depleted of pigment as long as the minus-end directed motor activity dominates.

Larger bodies, such as mitochondria, chromosomes and nuclei can be subject to a comparable translocation in the direction of aster centers. A case of more general biological significance is the movement that precedes the fusion of male and female pronuclei in fertilized egg cells. It depends on the formation of an aster that develops from the male-derived centrosome. In ctenophore eggs, where the thickness of the cytoplasmic layer varies between 5 and 30 μm, the large aster becomes disc-shaped, whereby its long diameter may reach up to several 100 μm [24].

The aster-like distribution of microtubules implies that, like in the dispersed state of chromatophores, plasma currents are generated along them by plus-end-directed motor activities. Interestingly, a kinesin-related motor activity has been found to be required for nuclear fusion in yeast [25]. While the microtubules grow along the female nucleus the limitation for optimal spreading of their distal ends leads to a local increase of the microtubule density (Fig. 3). This entails a superimposed outwards directed flow of cytoplasm that must be recycled through the microtubule-free space, whereby the nucleus is carried towards the

aster center. During the translocation the driving force and the resistance to the movement by decreasing intermicrotubular space grow in a differential manner until an equilibrium is reached at which the nucleus becomes stationary. The eventually occupied position equals the one of the male nucleus that is maintained, by the way, as a result the same streaming dependent equilibrium. Finally, the two flow patterns merge into one, which is determined by the lowest state of energy. In the final position the nuclei are pressed against each other, which may be of importance for the ultimate fusion process. In the case considered here the translocation is essentially restricted to the female nucleus. The reason for this is a lack of space for a substantial movement of the large aster in the thin cytoplasmic disc of the yolk-rich egg [24]. Obviously, a movement of both pronuclei towards each other, as observed in fertilized sea urchin eggs [26], implies that the mobility of the male nucleus-aster complex is less limited by available space. The vital role of the described nuclear translocation in egg cell fertilization is shown by the observation that no nuclear fusion occurs in Drosophila mutants that lack the male aster organizing center [27].

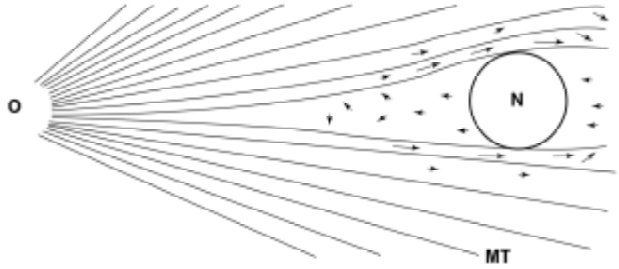

Fig. 3. Translocation of the female nucleus in the fertiziled egg by a sperm-derived aster. Deformation of the regular microtubule arrangement by the presence of the nucleus (N) results in locally increased microtubule densities. Arrows indicate the superimposed plasma flow owed to this redistribution. Symbols as in preceding figures. For explanation see text.

The same patterns of flow and movement can be envisaged to play a role in the accumulation of medium-sized chromosomes and organelles such as mitochondria and even small vacuoles in the polar spindle region in early mitosis [2,28]. Interestingly, the final distance from the aster center increases roughly with the particle size as expected by entrapment according to available intermicrotubular space.

CONCLUDING REMARKS

We have recently shown that activities of the various cellular motors generate plasma currents along microtubules and actin filaments [3]. This sheds a new light on the earlier assumption that particle movements in cells originate from

plasma streaming. Indirect evidence for plasma streaming in mitotic cells is indicated by birefringence measurements [29,30]. However, direct evidence has so far only been found in the very large internodial cells of Characeae [5,6], as most other cells are too small for such studies. It indicates, that the weak currents generated along individual actin filaments merge into a single strong stream by uniformly oriented filament bundling. Average velocities of particle movements in vivo are usually between 1 and 10 μm/sec, but may reach up to 50 μm/sec [4], while average velocities along single microtubules in vitro are usually below 1 μm/sec [31]. As is now shown in this study, the various streaming patterns that are obtained by such ordering of skeletal elements provide an explanation for a wide range of particle behaviours.

In the closed space of a cell any displaced cytoplasm and the particles present in it, must be recycled, even in the absence of additional skeletal elements. Such particle movements in the absence of properly oriented microtubules and actin filaments have been amply observed in mitotic spindles [2]. The recycling is an essential precondition for a steady particle flow. Notice that any particle translocation by some mechanical stepping mechanism [1] must cease as soon as all particles are accumulated at one bundle end, in the case of actin filaments at their membrane-attached barbed ends. If the particle recycling is supported by active streaming, such as generated by dynein activity in the properly arranged microtubules in a neuronal axon, it leads to recognizable forth- and back-shuttling and rotation of the particles [13] for reasons discussed above. Because of the strong friction in the thin tubes, axonal plasma flow and particle movements reach only about 10% of the velocity found along the characean actin bundles.

A particular property of the described system is that the steady state order of skeletal elements which determines the flow pattern is itself dependent on the motor activity involved. For example, in mitotic cells, where the kinesin-generated plasma streaming in traction fibers is towards the kinetochore-attached plus-ends of the microtubules, the recycling causes a strong bundling of the distal, free microtubule domains [8,9]. In contrast, in asters, where the flow is towards the free microtubule ends, it results in their maximal spreading. The situation is the same in the dermal chromatophores and causes together with the plasma recycling in the intermicrotubular space a forth- and back-shuttling of pigment granules, that leads to their spreading over the entire lobes. The reversal of the streaming pattern by changing from kinesin to dynein activity domination induces an immediate bundling of the distal microtubule domains [21,22], followed by an alternative flow pattern by which the pigment granules are trapped in the aster cores. It ought to be emphasized that an alteration of the streaming pattern in asters does not necessarely require a complete switch from one motor activity to the other. It is rather likely that both kinesin and dynein are simultaneously active, whereby the stronger activity determines the streaming direction. A hormonal signal that induces the activation or inactivation of one of

the two is then sufficient to cause an alteration of the distribution of pigment granules.

Another striking observation is that an excess of kinesin activity, which causes the dispersion of pigment granules in the asters of dermal chromatophores, induces a centripetal translocation of male nuclei in fertilized egg cells and of chromosomes and mitochondria in early prometaphase of mitotic cells. Here it is the size of the particles that entails a local microtubule redistribution and corresponding alteration of the flow pattern. These movements cease when the strength of the driving streaming forces match the obstruction forces that result from the high microtubule density. Thus transport by plasma streaming explains as well various types of particle movements as a temporal stable positioning by a dynamic equilibrium of forces.

It may be clear that no type of mechanical stepping mechanism can explain these various in vivo behaviours. The explanation becomes more complicated, however, when plasma streaming is not the only driving force, such as is the case in mitotic chromosome movement. This problem will be treated in more detail in a subsequent study.

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