TWO-DIRECTIONAL PATTERN OF MOVEMENTS ON
THE CELL SURFACE OF AMOEBA PROTEUS

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SUMMARY

Particles of latex, glass and precipitated Alcian Blue were studied cinematographically on the
surface of migrating Amoeba proteus and in the surrounding medium. The majority of the attached
and all unattached particles flow steadily forward in the direction of the endoplasmic streaming and
cell locomotion. Flow on the surface is faster than in suspension. Some particles stuck on the
membrane move backwards from the frontal region. This retrograde transport is slower than the
anterograde flow, and the rate decreases further when the particles approach cell regions adhering
to the substratum, accurately following the pattern of the withdrawal of ectoplasm in the same
zone. Both movements coexist in the same region and retrograde particles may pass anterograde
ones at a distance less than their diameter. Transition from forward flow to backward transport
occurs just behind the frontal cap, where the new ectoplasm is formed. The anterograde movement
is interpreted as reflecting the general forward flow of the laterally mobile fluid membrane
components, which become added to the frontal surface of the locomoting cell; the retrograde
movement as retraction of membrane components that, externally, are linked to the transported
material and, on the cytoplasmic side, to the contractile microfilamentous layer, as is postulated for
cap formation in tissue cells.

INTRODUCTION

The movement of particles on the surface of free-living amoebae has for a long
time been considered as an indicator of the behaviour of the cell membrane during
locomotion. All reports agree that such markers move generally forwards with
respect to the cell, but nevertheless accumulate in the tail region. Stockem (1966)
explained this paradox by stressing that forward transport of markers along lateral
pseudopodia does not coincide with the principal axis of cell locomotion.

The authors of the classical concepts of amoeboid movement usually considered
the membrane of amoebae as a quasi-permanent structure, which must be carried
along as a whole with the moving cell (Jennings, 1904; Mast, 1926; Griffin & Allen,
1959). They invoked some kind of rolling as its mode of transport. Goldacre (1961)
objected to the view that markers reflect the movement of the membrane, attributing
their transport to the electrophoretic forces generated between the poles of the cell.
He claimed that during locomotion the membrane remains stationary and is re-
circulated through the cell interior, being constantly ingested at the tail and re-
constructed at the front. This hypothesis was supported by Chapman-Andresen
(1964) and Jahn (1964) for amoebae and by Shaffer (1963, 1965) for the amoeboid
stages of cellular slime moulds. The presumption that membrane internalization at

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the posterior pole and renewal at the front are so rapid that they may be factors, or even the motor of locomotion, was later adopted in the interpretation of movements on the surface of fibroblasts (Harris, 1973; Bretscher, 1976, 1984).

In *Amoeba proteus* we found (Czarska & Grebecki, 1966) that granular markers move forward three-dimensionally (not by rolling) and that, moreover, a slight increase of the viscosity of the medium results in the manifestation of extracellular streamings of fluid, which follow the direction of movement of each pseudopodium. We advanced the view that the whole membrane is transported by its continuous unfolding on the surface of advancing pseudopodia and refolding on the contracting ones and on the tail. The folding–unfolding hypothesis has been later confirmed by cinematographic and electron-microscope studies (Stockem *et al.* 1969; Haberey *et al.* 1969; Haberey, 1971; Stockem, 1972) and supported by Wolpert (Wolpert & Gingell, 1968) in combination with his own (Wolpert & O'Neill, 1962; Wolpert, 1965) concept of the membrane flowing forward owing to lateral mobility of the lipid bilayer.

However, recent observations of ectoplasmic movements during locomotion (Grebecki, 1984, 1985) revealed that glass-wool hairs intercepted by amoeba during migration do not move generally forward. They are transported forwards by the withdrawing tail but backwards by the advancing frontal region, where the withdrawal of ectoplasm is seen along the fountain zone. This means that the hairs are transported from both body poles centripetally, but finally accumulate in the tail because the cell as a whole moves faster than its ectoplasmic layer. The resulting pattern may be compared with the capping, extensively studied in tissue cells (for reviews, see de Petris, 1977; Weatherbee, 1981; Oliver & Berlin, 1982; Geiger, 1983; Jacobson, 1983; Bourguignon & Bourguignon, 1984) and revealed also in amoebae (in *Naegleria gruberi* by King & Preston, 1977; and in *Chaos chaos* by Taylor *et al.* 1980).

It became necessary to re-examine in combined experiments these two apparently incoherent phenomena: the general forward flow of particles and fluid medium and the centripetal movement of glass hairs. The interest in them is increased by the recent profusion of controversial concepts of the mechanism of transport of surface markers and cross-linked surface receptors by different crawling and capping cells: the theories of surf-riding (Hewitt, 1979; Berlin & Oliver, 1982), bulk backward flow of the membrane (Abercrombie *et al.* 1970; Harris, 1973) or of membrane lipids (Bretscher, 1976, 1984), and the hauling of the complexed surface receptors by microfilaments (de Petris & Raff, 1973a,b).

The objective of the present report is to describe the general bidirectional pattern of the movement of markers on the surface of *A. proteus*, on the basis of cinematographic records, which will be later subjected to quantitative analysis.

**Materials and Methods**

Cultures of *A. proteus* were kept in the standard Pringsheim medium and fed twice a week on *Tetrahymena pyriformis*. Polytactic and monotactic forms (see Grebecki & Grebecka, 1978, for the terminology) were taken for experiments on the 2nd or 3rd day after feeding. Their motion was
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recorded in monochromatic green light (546 nm wavelength), in normal culture medium, on slides with glass spacers supporting the coverslip at a distance of 0-5 mm. The experiments were run in a semi-dark room, at 20 (±2)°C.

The following materials were used in suspension and, or, covering the surface of the slides: (1) Dow latex beads 1-009, 0-794 and 0-460 μm in diameter, diluted 1:1000 (which produced 0-01 % dry weight suspension according to the specification of the manufacturer); (2) crushed glass-wool hairs, 5–15 μm in diameter and 15–250 μm in length; (3) ground glass powder with particle sizes ranging from 1 to 10 μm; (4) Alcian Blue precipitate produced within 2–3 days in 0-04 % solution of the dye in the Fringsheim medium enriched with Ca²⁺ up to 2 mM, collected by sedimentation and resuspended in the culture medium (the supernatant did not stain the surface coat and failed to provoke any motor or endocytotic response by amoebae). All these particles were used either alone or in different combinations. Some other materials, such as carmine and Indian ink particles or calcium oxalate and inulin crystals, were occasionally used to repeat experiments reported by earlier authors.

A Biolar microscope equipped with a standard TV camera and differential interference optics of the Pluta system (made by PZO, Warsaw) were used with 20x/0-40 and 40x/0-65 planachromatic objectives. The image was recorded, either directly from the microscope or from the TV screen, by a Bolex H16 Reflex camera run at 4 or 1 frames per second by a combined Bolex and Robot time-lapse equipment. The films were reviewed with an LW International Mark IV Photo-optical Data Analyzer.

All the pictures shown in Figs 1-8 are prints of selected film frames, keeping stable reference marks outside the moving cells in a constant position.

RESULTS

Different locomotory forms of A. proteus were filmed in media containing 0-2–0-4 % methylcellulose in which minute crystals of inulin or calcium oxalate, carmine or Indian ink particles and latex beads, were suspended. The cinematographic records confirmed the earlier results (Czarska & Grebecki, 1966), demonstrating the three-dimensional forward flow of the viscous medium, parallel to the motor axis of amoeba. Further experiments proved, however, that an increase of viscosity is not necessary for the manifestation of this phenomenon. As shown in Fig. 1, latex beads suspended in the standard culture solution also regularly flow forward in the direction of cell locomotion, not only in contact with the cell surface but also at a distance of several micrometres from it. The exact transverse velocity profiles of suspensions flowing around moving amoebae have not yet been studied, but the present results clearly demonstrate that the latex particles adjoining the cell surface move forward considerably faster than those freely suspended in the medium (Fig. 1). It may also be easily estimated that the velocity generally decreases with distance from the cell (Fig. 4).

The film records demonstrated, moreover, that the extracellular flow is strictly dependent on the direction and velocity of endoplasmic streaming (i.e. on cell locomotion). It accelerates, slows down, stops or reverses its direction simultaneously with the endoplasm, when its flow changes either spontaneously or due to local stimulation of pseudopodial tips by white light or shade. The oscillating streaming of endoplasm provoked by 0-2 % ethanol is also reflected by the same oscillations in the extracellular flow of latex suspension.

The particles surrounding the cell or contiguous to its surface move forward with respect to external reference points, as well as to the cell itself, and thus they
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gradually approach the frontal zone. However, in the frontal pseudopodia of polytactic amoebae they never reach the extremity, but continue to move at a certain distance from the tip of the pseudopodium, until it is withdrawn. In monotactic amoebae, on the other hand, the extracellular particles overtake the advancing front and, as a result, retrograde countercurrents arise.

The apparently contradictory results obtained with glass-wool hairs (Grebecki, 1984, 1985), which move back from the front along the fountain zone, were again obtained in the present work. Film analysis of the backward movement of glass-powder particles, ranging from 1 to 10 μm in diameter, proved that their varying behaviour does not depend directly on their size, but rather on the nature of contact with the cell surface. Only glass particles stuck to the surface move backwards, whereas the others follow the general forward flow described before. Under a sufficiently large fragment of glass, as shown in Fig. 2, the development of an adhesive knob is observed, after which the particle starts to move back.

The backward movement from the front along the fountain zone is particularly well shown in suspensions of Alcian Blue precipitated by Ca²⁺ (Fig. 3). Ruthenium Red precipitates provide similar results. Clusters of those precipitates also adhere to specialized knobs (Fig. 4). The backward transport of glass particles and Alcian Blue precipitate is observed in both polytactic specimens (Fig. 2) and in monotactic ones (Fig. 3), and has the same features in both forms of amoebae.

The general forward flow of particles and the retrograde transport of some of them in close contact with the cell surface are not two alternative responses that exclude one another, but may coexist. Fig. 4 shows an aggregate of Alcian Blue moving backwards against the forward flow of the surrounding latex suspension. The same pattern of two simultaneous opposite movements has also been recorded in mixtures of glass powder with latex and in pure suspensions of glass powder or precipitated Alcian Blue. Even the latex beads closely adjoining the cell surface form, in fact, two populations. Most of them move forwards as described before, but a few are transported backwards. Two latex beads when moving in opposite directions, as those shown in Fig. 5, may cross each other’s path at very short distances, which may be less than their diameter (<1 μm). Such measurements are possible when the two opposite movements are recorded on the upper surface of an amoeba.

Fig. 1. Forward flow of latex beads around polytactic amoeba, on the cell surface (white arrowheads) and several μm from it (black arrowheads), demonstrated by five film frames (A–E) selected at 4-s time intervals. Note that the movement on the surface is considerably faster than in suspension. Large white arrows inside the cell indicate (in Figs 1–8) the frontal direction of endoplasmic streaming. Bars (Figs 1–2, 4–9), 10 μm.

Fig. 2. A crumb of glass (black arrowhead) intercepted by the tip of a polytactic pseudopodium (A), rearrangement of its position just behind the frontal cap (B–C) and its further transport backwards along the pseudopodium, which continues to advance (C–E). Note that in C–E the particle is kept by an adhesive knob; 10-s intervals between the successive stages.

Fig. 3. Interception of a clump of Alcian Blue precipitate (black arrowhead) by the front of monotactic amoeba (A) and its transport backward upon the cell surface (B–C). Pictures taken at 24-s intervals. Bar, 100 μm.
Fig. 4. Three stages of the backward transport of a clump of precipitated Alcian Blue (black arrowhead) with the simultaneous forward flow of latex beads suspended in the surrounding medium. Latex is shown as two constellations of particles moving generally forwards but changing their configurations because the velocity decreases with distance from the cell surface. Note the adhesive knob under the clump of Alcian Blue; 10-s intervals between the pictures.

Fig. 5. Two neighbouring latex beads on the cell surface moving in opposite directions, forward (black arrowheads) and backward (white arrowheads), shown in A–D at 2-s intervals.

Fig. 6. Latex particle on the cell surface (white arrowhead) transported back from the front of amoeba strictly in unison with the ectoplasmic granules and the basis of a lateral pseudopodium (black arrowheads), which are withdrawn in the same direction. Pictures taken at 2-s intervals.

Fig. 7. Forward movement of a group of latex beads on the cell surface (white arrowheads), independent of the backward movement of ectoplasmic inclusions (black arrowheads), shown in the frontal part of a monotactic amoeba at intervals of 4 s.
The most striking feature of the backward movement of particles on the surface of an amoeba is its precise coordination with the movement of ectoplasmic inclusions on the opposite, cytoplasmic side of the cell membrane, which is observable with all suspended materials applied in this study. The extracellular retrograde transport is from the beginning slower than the extracellular forward flow; it gradually slows down further and eventually stops at the middle body region, where the cell is firmly attached to the substratum and the ectoplasm comes to a halt. The backward movement of extracellular particles is detected only in those cell regions and situations that favour the withdrawal of ectoplasm in the form of the fountain movement. The coordination of movements on both sides of the cell membrane is particularly easy to record when latex is used as marker. Fig. 6 demonstrates that a latex bead transported backwards does not change its position with respect to the underlying ectoplasmic inclusions, but moves strictly together with them. Extracellular particles moving in a retrograde direction also keep a constant position with respect to small lateral pseudopodia, hyaline blebs and other elements of the cell contour, which also move backwards together with the withdrawing frontal segment of the principal ectoplasmic cylinder. Fig. 7 shows that, in contrast, latex moving forwards in the same cell region, though apparently in contact with the cell surface, does so independently of the behaviour of the ectoplasmic layer.

In polytactic amoebae particles are usually transported backwards if they are picked up by the frontal tip of a pseudopodium during its progression (Fig. 2). This also occurs in monotactic forms to those particles that reach the front after a phase of flowing forwards (Fig. 8). Their forward movement stops usually just behind the frontal cap, in the zone where new ectoplasm is being formed from the endoplasm. There the halted particle may be repositioned, often being rotated, and then transported backwards in unison with the ectoplasmic layer. The change of direction of the glass particle in the endoplasm–ectoplasm conversion zone of a monotactic amoeba is shown in Fig. 8. Many examples of exactly the same behaviour were recorded with different kinds of particles, including latex beads.

The retrograde ectoplasm-dependent transport of a particle may cease at any moment and then it immediately joins the others that flow over the cell surface forwards. Otherwise, the withdrawing particles reach the region where the ectoplasm is stationary, stop there, are gradually incorporated into the tail part of the amoeba and eventually shed into the medium. It seems relevant that sometimes the ectoplasm-dependent backward transport of a latex bead down to the tail of a monotactic cell may be terminated by its endocytosis. As demonstrated in Fig. 9, the bead is engulfed by hyaline micropseudopodia developed in the constriction preceding the uroid, that is close to the zone that is also known (Wohlfarth-Bottermann & Stockem, 1966) to be a site of permanent pinocytosis in amoebae.

**DISCUSSION**

It may be concluded that the behaviour of foreign particles around and upon the cell surface of a migrating amoeba is composed of their anterograde and retrograde
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transport, which are two distinct types of movement driven by different mechanisms, though they can coexist in the same cell regions and at the same time. In spite of their differences, both movements seem to be generated locally and everywhere along the cell surface, and thus reflect the intrinsic movements of two different fractions of the surface material. As far as the retrograde component is concerned, this conclusion is strongly supported by strict coupling of the movements of extracellular particles and ectoplasmic granules on the two opposite sides of the plasma membrane. It seems less obvious in the case of the anterograde transport of particles, especially those lacking direct contact with the cell. It might be supposed that they are pulled, owing to the viscosity of the medium, by the advancing front of the amoeba, or driven by any force developed at one or both body poles (as in the electrophoretic hypothesis of Goldacre, 1961). However, any movements not generated locally would be forcibly hampered close to the membrane by friction. The fact that the highest speed of particles occurs in contact with the membrane and their velocity declines with distance from the membrane indicates that the driving force is generated everywhere locally by the cell surface, which itself moves forward.

Coexistence of two opposite movements within the cell membrane (and the surface coat) of amoeba is fully conceivable in view of the lateral fluidity of membrane lipids, postulated long ago for amoeboid cells by Wolpert (1965), and now commonly accepted as a part of the fluid mosaic model of membrane structure and function (Singer & Nicolson, 1972). The fact revealed by the present experiments, that two latex beads moving in opposite directions may cross each other at a distance less than 1 μm, clearly demonstrates the high fluidity of the cell surface material in A. proteus.

Probably the membrane lipids, with those proteins that are not anchored to cortical microfilaments, flow forwards, pulled by the advancing front due to the internal 'microviscosity' of the lipid bilayer. That seems to be the only mechanism capable of accounting for the demonstrated correlation between endoplasmic streaming and the extracellular anterograde flow. The stock of material ready to flow forwards is perpetually liberated from the posterior surface areas by their gradual unfolding and regenerated by refolding the surface of retracted pseudopodia (Czarska & Grebecki, 1966).

The function of the general forward flow of the cell membrane in a moving amoeba is to supplement the surface material in the anterior part of the body as necessitated by changes in its shape and position. These changes are too fast in amoebae to be

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Fig. 8. Transition from the forward flow to the backward transport in monotactic amoeba. The particle of glass powder (black arrowhead) approaches to the front in A–C, is held and rotated at the endoplasm-ectoplasm conversion zone in D, and transported backwards in E–H. Intervals of 10 s separate the successive stages.

Fig. 9. Endocytosis of a latex bead (white arrowhead), which was transported backwards along the surface of monotactic amoeba up to the basis of the uroid. and in A show, respectively, the uroidal and frontal directions. Note the hyaline micropseudopodium, which keeps the latex particle in A and retracts with it in B–C. A much larger hyaline pseudopodium develops in D–E, and engulfs the bead in F by invagination. Irregular intervals between the successive pictures; A–C stages are repetitive and may last 15–100 s, D–F stages took in the recorded case 6 s.
compensated for by intracellular membrane turnover, as was demonstrated by immunofluorescence techniques (Wolpert & O'Neill, 1962) and by the study of the dynamics of permanent endocytosis (Stockem, 1972). Therefore, the membrane is recirculated between the tail and the front of locomoting amoeba, at the cell surface.

As to the retrograde component of extracellular movements, it may be postulated that a particle is transported backwards if the surface receptors responsible for its adhesion to the cell become (or were) attached to the submembranous layer of microfilaments. Direct demonstration of such a linkage between a backwardly moving particle and the cortical ectoplasm is still lacking, but some present observations strongly suggest its existence: (1) the retrograde transport is manifested only in association with the withdrawal of the ectoplasmic layer in the fountain pattern; (2) the particles move backwards precisely together with the ectoplasmic inclusions on the other side of the cell membrane; (3) they move back in synchrony with small lateral pseudopodia and other elements complicating the cell contour; (4) the backward movement, contrary to the forward one, is possible only in contact with the cell surface; (5) adhesive knobs may be seen by light microscopy under the larger particles during retraction; (6) some retracted particles are eventually endocytosed by invagination, which is generally recognized as depending on contraction of cortical microfilaments (Allison & Davies, 1974; Klein & Stockem, 1979). Moreover, the existence of a link between the activated surface receptors and the contractile submembranous layer has been demonstrated in other cells, when patches of receptors cross-linked by lectins, antibodies or other ligands move back to form caps (Edelman, 1976; de Petris, 1977; Bourguignon & Bourguignon, 1984).

It is proposed in consequence that some particles on the surface of amoeba are kept there by adhesion, i.e. are attached to the contractile apparatus. Therefore, they accurately follow all movements of the ectoplasmic cylinder as described here and before (Grebecki, 1984, 1985), independently of the unfixed fluid surface material, which flows over them in the frontal direction.

The retrograde transport of particles on the surface of *A. proteus* presents some common features with the saltatory motion of particles on the surface of reticulopodia of a foraminiferan *Allogromia*, though the first seems to depend on microfilaments and the second on microtubules. The latex beads adhering to reticulopodia also manifest phases of motion accurately coupled with the movement of intracellular organelles (Bowser et al. 1984); their saltation ceases if the cytoskeleton–membrane contact is broken, and a link between extracellular particles and the cytoskeleton is demonstrated in detergent-extracted reticulopodia (Bowser & Rieder, 1985).

Other analogies appear in respect of the backward movement of foreign particles on the surface of fibroblasts (Ingram, 1969; Abercrombie et al. 1970; Harris, 1973; Albrecht-Bühler, 1973) and growing axons (Bray, 1970), as well as of the segregation of receptor–ligand complexes in the course of capping (cf. de Petris, 1977; Bourguignon & Bourguignon, 1984). In fibroblasts a retrograde transport of particles is seen on the surface of a lamellipodium, in front of the firm cell-to-substratum attachment zone, i.e. where in amoebae the fountain movement may arise; it also slows down gradually *en route* from the leading edge to the cell centre; it is also well
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correlated with backward motion of the outer cell contour (ruffles and blebs) and "a generalized backward movement" occasionally seen inside the lamellipodia (Abercrombie et al. 1970; Harris, 1973).

Nevertheless, the bulk backward membrane flow proposed as the mechanism of movement of markers upon the surface of fibroblasts by Abercrombie et al. (1970) and Harris (1973), as well as the backward lipid flow postulated by Bretscher (1976, 1984), are inadequate to explain the present results. In A. proteus a general flow of the surface material, attributed mainly to its lipid fraction, is evident but directed forwards. Neither of the surf-riding models (Hewitt, 1979; Berlin & Oliver, 1982) seems applicable, because of the lack of any system of surface waves in amoeba that could account for the simultaneous transport of neighbouring particles in two opposite directions.

On the contrary, the mechanism proposed here for amoeba is fully in line with the theory put forward to explain the capping of cross-linked receptors by de Petris & Raff (1973a,b), admitted by Harris (1973) as an "alternative hypothesis", and adopted by many followers (cf. Bourguignon & Bourguignon, 1984). According to this theory, patches of receptors are linked to the contractile apparatus and hauled backwards by it. Moreover, as stressed both by de Petris & Raff (1973b) and Harris (1973), such a model necessitates compensatory countercurrents in the capping cells. The present work shows that in A. proteus such a countercurrent actually exists in the form of a general forward surface flow, at least during locomotion.

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