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# The role of cytoplasmic streaming in symplastic transport

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## ABSTRACT

**The distributing of materials throughout a symplastic domain must involve at least two classes of transport steps: plasmodesmatal and cytoplasmic. To underpin the latter, the most obvious candidate mechanisms are cytoplasmic streaming and diffusion. The thesis will be here advanced that, although both candidates clearly do transport cytoplasmic entities, the cytoplasmic streaming *per se* is *not* of primary importance in symplastic transport but that its underlying molecular motor activity (of which the streaming is a readily visible consequence) *is*. Following brief tutorials on low Reynolds number flow, diffusion, and targeted intracytoplasmic transport, the hypothesis is broached that macromolecular and vesicular transport along actin trackways is both the cause of visible streaming and the essential metabolically driven cytoplasmic step in symplastic transport. The concluding discussion highlights four underdeveloped aspects of the active cytoplasmic step: (i) visualization of the real-time transport of messages and metabolites; (ii) enumeration of the entities trafficked; (iii) elucidation of the routing of the messages and metabolites within the cytoplasm; and (iv) transference of the trafficked entities from cytoplasm into plasmodesmata.**

*Key-words*: cytoplasmic streaming; diffusion; endoplasmic reticulum; intracellular transport; molecular motors; plasmodesmata; trafficking.

## INTRODUCTION

The green plant, like an army, has two all-consuming activities, without which it can not hope to prevail. The first of these is *logistics*. The second is *command*, *communication*, and *control* ($C^3$). Because the topic of this is essay cytoplasmic (protoplasmic) streaming in the context of symplastic transport, these two imperatives will be treated in the context of a cell whose contents are convecting.

*Logistics* is the art/science of getting the supplies needed to the unit (a cell) and distributing them appropriately. in the context of the cell. This means: (i) taking them up, either across the plasmalemma from the apoplast or from the protoplasm of a neighbouring cell by way of connecting plasmodesmata; and (ii) distributing them appropriately within the protoplasm. Our focus will be upon distribution.

$C^3$ is the art/science of gathering reliable information and then transmitting unambiguous orders to appropriate effectuators. In the context of the cell, which participates in a far more decentralized command structure than an army, this means: (i) carrying out its programmed activities; (ii) sensing its surroundings and its internal state and transmitting appropriate information and/or commands; and (iii) receiving and effecting commands from elsewhere in the plant.

None of this is simple. and all of this must be done in a complex three-dimensional ultrastructural milieu which does not conform to the diffusion-dominated 'watery bag' model of yesteryear (Hochachka 1999). To paraphrase the elder Moltke, 'in [physiology] with its enormous friction even the mediocre is quite an achievement' (van Creveld 1985, p.13). Or to cite from Polybius (a soldier/historian of the 2nd century BC) a rather more direct parallel between war and biology: 'nature makes a single trivial error sufficient to cause failure in a design, but correctness in every detail barely enough for success' (van Creveld 1985, p. 264). That the plant fares very well indeed speaks volumes for the robustness and resilience of its strategies.

In the distribution of both supplies (logistics) and information ($C^3$) the plant has many options, including:

1 *Convection*. This form of mass transport is, in plant biology, typified by cytoplasmic streaming. If the supplies are metabolites, they will certainly be carried along by the motion of the cytoplasm. If the information is encoded in chemical messengers, it too will be carried along by the motion. However, the transport will be only along the streamlines of the flow: convection will never move metabolites or chemical messengers perpendicular to the flow. Convection is presently considered to be a result of endoplasmic 'molecular motors' running along actin microfilaments or possibly along microtubules (Reddy 2001). But it is an open question whether the convective flow (i) constitutes a major payoff arising from this motile activity; or (ii) is a byproduct of lesser utility which arises from drag on the entities towed by the moving motors. That is, how is the utility to the cell of motor motion to be apportioned between (a) stirring of the cytoplasm; and (b) siting of the towed entity at a new location; presumably a cost function exists and is reflected by plant evolution, even though we do not at present know how to formulate it.
2 *Diffusion*. This reflects the kinetic motion of the molecules comprising the cytosol. As a result of intermolecular collisions, a molecule in a particular compartment of the cytosol will execute a random walk and will, other influences being absent, tend in time to assume a uniform probability distribution within the compartment (cf. Saha

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& Srivastava 1950 and van Kampen 1981). Diffusion can move metabolites and messengers perpendicularly to the streamlines of cytoplasmic flow.

3 *Tow*. This denotes a motive force imposed differentially upon certain classes of entities in a medium. For example, the motion of electrons and holes in a semiconductor is a 'tow' phenomenon called *drift*. Magnetophoretic sedimentation of amyloplasts by a non-uniform magnetic field (e.g. Kuznetsov & Hasenstein 1996) is likewise a 'tow' phenomenon; and so also would be selective coupling of myosin to some particular type of macromolecule (or vesicle) and the subsequent translocation of the macromolecule along actin trackways. Obviously, when the towed entities are dragged through a fluid, convection will result. Obviously also, tow may be mediated either by fields ('action at a distance') as in sedimentation and magnetophoresis or by direct mechanical linkage as in molecular motor based processes.

4 *Electrical*. This could be electrophoretically driven mass flow, but there is little evidence that this is important on an intracellular level; and, were it to exist, it should be highly wasteful of metabolic energy because the cytoplasm is very close to electrical neutrality (cf. Pickard 1965). Or it conceivably might be electrically mediated information transfer, except that long-distance nerve-like signalling is uncommon in plants and intracellular information transfer by electrical means has not been shown to exist (e.g. Pickard 1973; 2001).

5 *Pressure*. On the whole plant level this could include signals such as pressure disturbances (Malone 1996). On the cellular level, the focus of this review, this could be manifested either by pole-to-pole pressure driven mass flow coupled to plasmodesmata or by transcellular osmosis; as this possibility has not been extensively studied, it will be largely neglected.

This leaves the first three items of the list as the prime candidates for mediating the steps of symplastic transport which occur within an individual cell. They will be discussed one by one in subsequent sections.

But before proceeding to these discussions, it is essential to clarify what one means by 'symplastic transport'. In 1879 Eduard Tangl observed intercellular '*Strängen*' *(*cords or veins) between plant cells and promptly realized that these '*Verbindungskanälen*' *(*connecting passages) might enable the plant to coordinate the activities of its individual cells (cf. van Bel & Oparka 1995). This notion of a coordinated syncytial protoplasm gradually evolved, with the term 'symplast' being coined in 1880 by von Hanstein (cf. Oxford English Dictionary http://dictionary.oed.com/cgi/entry/00245169), the term 'plasmodesmata' being first applied to Tangl's passages in 1901 (Strasburger 1901), and the contrasting terms 'apoplast' and 'symplast' being used together in 1930 (Münch 1930; p. 73). At present, *sensu stricto*, these last two terms mean, respectively: a plant domain (or set of domains) which is entirely outside the plasmalemmal continuum; and, conversely, a set of plant domains (or a domain) which are entirely inside the plasmalemmal continuum. The symplast is cytoplasmic; the apoplast is the cell wall and beyond. But somehow, in common usage, 'symplastic transport' only peripherally includes transport in the phloem. Perhaps an adequate way of putting it is:

'Symplastic transport' denotes the transport and distribution of molecules in general and chemical messengers in particular within the cytoplasm of a symplast domain of nonvascular cells and the spreading of these molecules from cell to cell via plasmodesmata'.

The concept of 'symplast domain' is well discussed by van Bel & Oparka (1995).

Moreover, the apoplast no longer appears as inert and uninteresting as it once did and is increasingly implicated in storage, transport, and reactions (cf. Sattelmacher 2001). In addition, although the bulk of the mole-miles racked up in symplastic transport must occur in the cytoplasm, yet nevertheless satisfactory transport still depends vitally upon such processes as *trans*-plasmalemmal water flux, plasmodesmatal function, and phloem transport. That is, long-distance transport in plants must be viewed as an integrated system-wide problem of logistics in which issues of command and control are unavoidable, even if not clearly understood at present.

## CONVECTION: MASS FLOW AT LOW REYNOLDS NUMBER

Convection within the cytoplasm is called 'cytoplasmic streaming' (or 'protoplasmic streaming'); and its discovery is attributed to Bonaventura Corti (e.g. Pfeffer 1906, Kamiya 1959). It has been reviewed repeatedly, for example by Ewart (1903), Pfeffer (1906), Kamiya (1959, 1960, 1981), Britz (1979), Seitz (1979), Kuroda (1990), Shimmen & Yokota (1994), Grolig & Pierson (2000), and Staiger (2000).

That streaming in the plant cell is of key importance is graphically borne out by the classical observation (Hofmeister 1867; Fig. 10) that, if some care is taken, a cell can be plasmolysed without disrupting the streaming. This observation has been validated repeatedly, was made by Ewart (1903; pp. 8–9) in his monograph, has been reviewed by Kamiya (1959; pp. 96–98), is still being commented upon in the literature (Kurkdjian *et al*. 1993), and has by the author been observed in *Chara* desiccating at the margin of a pond whose water level had been precipitously lowered. In the Characeae, its existence is so essential that Ewart has remarked (Ewart 1903; p. 4) 'it is so closely connected with vitality . . . that permanent cessation always indicates a fatal injury'; and this has been borne out by author's incidental experience in a variety of electrophysiological studies (e.g. Barsoum & Pickard 1982). These observations attest to a marked positive association between intracellular motility and survival of the cell, although there is as yet no widely accepted enumeration of the benefits which this motility confers upon the cell (cf. Pickard 1972, 1974).

Cytoplasmic streaming *is not* the result of body (i.e. ponderomotive) forces acting on the ground cytosol but rather a manifestation of the differential motion of the surfaces bounding the cytosol (cf. Kamiya 1981). This simple idea is however, apt not to be obvious to someone conditioned by the high Reynolds number fluid dynamics of flowing streams, pouring wine, or briskly agitated laundry. In these three examples, the kinetic energy of the fluid dominates strongly: it does not do so at the subcellular level.

First, absent phenomena presumed rare or inconsequential at the subcellular level such as porous boundaries (cf. Chellam, Wiesner & Dawson 1995) or slip boundary conditions (e.g. Allison 1999), the empirically observed nature of the liquid–solid interface dictates imposition of classical no-slip boundary conditions: along a liquid–solid interface, the velocities of the two phases are equal (e.g. Milne-Thomson 1950, sect. 19·05). This means that, if a surface moves within the cellular coordinate system, it drags the adjacent cytosol with it; that is, the cytosol near a moving structure (membrane or organelle) tends to move with that structure.

Second, the nature of the fluid dynamic phenomena manifested in a system depends strongly upon the system's Reynolds number

$$R = \rho U a/\eta, \tag{1}$$

where $\rho$ (kg m$^{-3}$) is the cytosolic density (approximately 1000), $U$ (m s$^{-1}$) is a velocity characteristic of the streaming motions ($\lesssim 100 \times 10^{-6}$), $a$ (m) is a length characteristic of ultrastructural dimensions ($\lesssim 10 \times 10^{-6}$), and $\eta$ (Pa s) is the effective viscosity of the cytosol ($\gtrsim 1 \times 10^{-3}$). Thus, for cytoplasmic streaming, $R \lesssim 10^{-3}$ (dimensionless); and this ensures (i) that viscous effects will outweigh kinetic energy effects; and (ii) that the approximations of low Reynolds number flow will obtain. For the author, this has meant that the only way he was able to achieve even marginal familiarity with the counterintuitive behaviours of these flows was by viewing repeatedly G. I. Taylor's remarkable short film *Low-Reynolds-Number Flows* (Taylor 1967).

Third, low Reynolds number flow arising from the towing of cytoplasmic structures or molecules does *not* mix the cytoplasm in the sense that dragging a perforated spoon through a kettle of soup mixes the ingredients. One can, however, get an inkling of the difference if such flow from everyday experience by comparing the result of (i) plunging a soiled butter-knife into a jar of honey and stirring once (low Reynolds number flow); and (ii) lowering a spoonful of cream into a cup of tea and stirring once (high Reynolds number flow): in the former case viscosity dominates and one is left with a semi-permanent streamline of butter and crumbs suspended in the honey; whereas in the latter case kinetic energy dominates, tumbling turbulence and/or vortex flow results, and the cream mixes rather thoroughly into the tea. In particular, low Reynolds number flow manifests 'kinetic reversibility' (Taylor 1967) which can be illustrated with the following experiment. Let a blob of thick coloured syrup be injected into a coaxial container of clear syrup; diffusion aside, it will be observed to sit there without mixing. Next let the outer cylinder be slowly rotated causing the blob to elongate markedly; the elongated blob will also tend to persist. Finally, let the rotation of the outer cylinder be *precisely* reversed and observe that the blob resumes its original shape!

Fourth, because the Reynolds number is so small, streaming and/or cytoplasmic mixing due to aperiodic mechanical agitation (e.g. sloshing) seem unlikely to be important.

Cytoplasmic streaming *is* the result of the action of 'molecular motors' which ratchet along cytoskeletal cables dragging various organelles and neighboring cytosol with them (Kamiya 1981; Kachar & Reese 1989; Williamson 1993; Shimmen & Yokota 1994; Reddy 2001). The cables thus far implicated in subcellular motion are composed either (i) of actin microfilaments (an actin polymer commonly called F-actin) roughly 5–9 nm in diameter, or (ii) of microtubules (an $\alpha/\beta$ tubulin polymer) roughly 25 nm in diameter (Reddy 2001). The macromolecular 'engines' which are by ATP-hydrolysis propelled along these fibrillar trackways are: for actin microfilaments, myosins (Yamamoto, Hamada & Kashiyama 1999; Cai, Del Casino & Cresti 2000; Shimmen *et al*. 2000; Kashiyama *et al*. 2000; Yokota 2000), members of a large pan-eukaryotic superfamily (Mermall, Post & Mooseker 1998); for tubulin microtubules, either kinesins or dyneins (Reddy 2001; Cai *et al*. 2000; Dole *et al*. 2000), also widely distributed among the eukaryotes. Of these motor systems, the actin-myosin seems at present to be by far the most important for cytoplasmic streaming (Shimmen & Yokota 1994); and dynein homologues appear to be absent from the *Arabadopsis* genome (Lawrence *et al*. 2001).

Based upon the trajectories of protoplasmic granules, Kamiya (1959; chs. I & II) classified the readily visible streaming in cells of the green plants into five (overlapping) groups: (i) *agitation*, in which the individual granules have a significant, subjectively stochastic component superimposed upon their fairly steady tow velocities; (ii) *circulation*, in which the individual granules move in a rather more predictable fashion even though neighbouring granules may manifest quite different motions; (iii) *streaming along definite tracks*, in which granule motion is confined to numbers of (mostly parallel) courses whose velocities of flow seem rather uncorrelated; (iv) *fountain streaming*, in which the motion is one direction along the central axis of the cell and the other direction along the cell's periphery; and (v) *rotational streaming*, in which the motion is up one side of the cell (often with a tendency to spiral) and down the other. These distinctions are more readily comprehended by studying the diagrams of streaming in pollen tube protoplasm provided by Iwanami (1956). Across the Viridiplantae, *visible* streaming (when present) has speeds roughly within the range 1–100 $\mu$m s$^{-1}$, with 10 $\mu$m s$^{-1}$ being perhaps typical (Kamiya 1959; Table 1). However, there is no essential requirement that the streaming be visible to the casual (or even dedicated) observer because slow motion of a single motor may not cause sufficient optical inhomogeneity to be detected. Thus, a reasonable definition of 'cytoplasmic streaming' is:

Any cytosolic convection arising from the action of molecular motors moving along cytoskeletal cables.

This definition emphasizes the active metabolically driven nature of the process. and the low Reynolds number environment mandates that streaming stop dead in its tracks when motor activity ceases. The most familiar (and perhaps the most important) realization of this process is the towing of subcellular organelles along actin trackways by myosin motors; but motions along microtubules and other cables are also included.

The best known of the above types of streaming is rotational because it is so readily studied in the giant cells of the Characeae (Kamiya 1959, 1981). Such streaming is somewhat atypical in that: (i) it is steady, whereas in putatively more advanced plants flow is much more variable with some cells not streaming visibly for prolonged periods (Ewart 1903; Shimmen & Yokota 1994); and (ii) the entire cytoplasm moves in a coordinated fashion whereas streaming in seemingly erratic directions along cytoplasmic strands (often transvacuolar) seems more characteristic of higher plants (e.g. Hofmeister 1867). However, the very simplicity of the streaming in charophytes (with its uniformly moving peripheral cytoplasm, its central vacuole, and its fixed 'indifferent lines' between the streaming zones) made it possible to model the flow hydrodynamically and to effect a comprehensible closed form solution which adequately predicted both its radial and its angular variations (Pickard 1972). As this model yielded accurate granule velocities in both cytoplasm and vacuolar sap but included no mention of the tonoplast, an obvious possibility was that the tonoplast shears like a fluid. At the time, this seemed mysterious. But viewed from the perspective of the fluid mosaic model of biological membranes (Singer & Nicholson 1972; De Weer 2000), it seems only logical because it is known from monolayer models of membranes that such structures can be have area–pressure and area–surface-potential isotherms with gas-like virial behaviour (Sehgal, Pickard & Jackson 1979). Presumably, such behaviour could be true of cellular lipid bilayers in general. Moreover, the endoplasmic reticulum appears capable of undergoing shear and remarkable metamorphoses of macrostructure, presumably without functional impairment (Kachar & Reese 1989; Lichtscheidl & Baluška 2000). Nevertheless, 'relatively little is known about the biogenesis, dynamics, maintenance, and inheritance of the organelle itself' (Powell & Latterich 2000).

### Summary

The points that should be retained from this brief overview of cytoplasmic streaming are:

1 Streaming is an active process driven by ATP-powered molecular motors.
2 These motors ratchet along fibrillar trackways in the cytoplasm, most often pulling a variety of attached entities with them.
3 These entities in turn drag the ambient cytosol with them producing the streaming.
4 Streaming is a low Reynolds number phenomenon and does not therefore produce efficient mixing of the cytosol.
5 Streaming may also shear the endomemranes, apparently without causing significant harm.

## DIFFUSION AND ITS LIMITATIONS

As summarized by Hochachka (1999), cellular physiology historically has embraced two major schools of thought regarding models of cell function and regulation: the one 'assumes that cell behavior is quite similar to that expected for a watery bag of enzymes and ligands'; whereas the other 'assumes that three-dimensional order and structure constrain and determine metabolite behavior'. The first emphasizes near equilibrium conditions and diffusive transport; the second stresses subcellular structure and molecular motors. and the first is increasingly under siege (e.g. Wheatley & Malone 1993; Reuzeau, McNally & Pickard 1997; Hochachka 1999; Agutter & Wheatley 2000; Luby-Phelps 2000).

However, it can not be that diffusion is irrelevant within the cytosol. First, convection alone at low Reynolds number will not spread a blob of metabolite uniformly throughout the cytosol (cf. Taylor 1967): it will merely generate a complicated but deterministic three-dimensional pattern within the cytosol. Second, ionic mobility is related to ionic diffusivity by way of the Einstein relation, which is very general and should hold given only the applicability of Maxwell–Boltzmann statistics to the charged entity (Ashcroft & Mermin 1976); therefore, because the cytoplasm is well known to be electrically conducting (e.g. Cole 1968), it can be presumed that small particles diffuse within it. Moreover, direct measurements of electrical conductance have been made on gelled electrolyte solution, a cytoplasm surrogate; and the effect of gelation on conductance is minor (Ewart 1903; pp. 123–126). Third, relative diffusion coefficients of various large molecules in cytoplasm have been measured (cf. Luby-Phelps 2000) and shown to be smaller than in aqueous solution but definitely not zero.

Each of the two outlooks described above has particular benefits for particular studies, and it seems obvious that biological reality must incorporate both to achieve a robust synthesis: the cell *is* packed with macromolecules, cables, and endomembranes which *do* affect intracellular transport, both *actively* and passively; but that this merely slows the free diffusion of solute particles, both by general tortuousity of pathway and by specific steric hindrance. That is, neither molecular motors alone nor diffusion alone can meet the transport needs of cytoplasm or symplasm; and this view seems now to be ascendant.

Diffusion, as it appeared relevant to plant biology 45 years ago, was beautifully summarized by Spanner (1956). As relevant to biophysicists and biochemists, it has been

treated by Stein (1986) and by Tanford (1961); and, as a mathematical model of nature, the classical diffusion (either of molecules or of heat) was well handled in the treatise of Carslaw & Jaeger (1959). These aspects will not be emphasized because most modern plant biologists have a pretty good intuition for what diffusion does. Nevertheless, there are several aspects of diffusion which are often neglected although physiologically important.

## Few-particle diffusion

It has been forcefully pointed out (e.g. Luby-Phelps 2000) that the reaction volumes of subcellular compartments can be very small and may, at physiological concentrations, contain so few copies of a particular reacting species that the continuum predictions of diffusion–reaction theory give way to stochastic fluctuations.

Nevertheless, there are still attractive arguments for continuing to make putatively plausible estimates with the formalism of classical continuum diffusion. First, our knowledge of cell biology seems at present much too sketchy to create of a demonstrably better alternative. Second, the differential equations of diffusion are linear in particle concentration, implying that the particles do not interact. Hence, if a numerically large but geometrically compact swarm of particles is suddenly released in a liquid, each particle pursues its random walk independent of the others. and therefore the prediction of the diffusion equations must represent a simple sum over the entire ensemble of 'walking' particles. That is, the concentration distribution of the diffusion equations is also the probability distribution for the location of a single particle at some moment after release.

The author calls this notion the 'ergodic heuristic'. He makes no claims for its universal, rigorous validity. But he expects that, if you average particle distribution within a single 10 nm vesicle over time or within a large ensemble of vesicles at a particular instant of time, you will find that both are close (a) to each other; and (b) to the predictions of continuum diffusion. In short, he believes that simple diffusive predictions should be quite good enough given the uncertainties of present knowledge and the reality that the subcellular environment is doubtless not the time-invariant milieu postulated by most modellers.

## The Stokes–Einstein formula

The Einstein relation between the diffusion coefficient $D$ ($m^2$ $s^{-1}$) and the mobility $\mu$ [$m^2$ $(V{\cdot}m)^{-1}$] of an ion is (Ashcroft & Mermin 1976)

$$D/\mu = k_B T/q, \tag{2}$$

where $k_B$ (J $K^{-1}$) is the Boltzmann constant ($1{\cdot}380 \times 10^{-23}$), $T$ (K) is the absolute temperature, and $q$ (C) is the net charge on the ion. If the ion is idealized as a sphere of effective radius $a_{eff}$ (m) undergoing low Reynolds number electrophoresis at a velocity $\mu\mathbf{E}$ in a vector electric field $\mathbf{E}$ (V $m^{-1}$), the drag it experiences will be just (cf. Robinson & Stokes 1959) $6\pi\eta a_{eff}\mu\mathbf{E}$. This force must be balanced by the Coulomb force $q\mathbf{E}$ on the ion, yielding via Eqn 2, the Stokes–Einstein formula

$$D = \frac{k_B T}{6\pi\eta a_{eff}} \tag{3}$$

where $a_{eff}$ is commonly known as the Stokes radius; also, it should be remembered from Eqn 1 that $\eta$ is an effective viscosity.

Because, roughly, the volume of a particle should scale linearly in its effective molecular weight $M$ [$u = Da$] (cf. Tanford 1961; sect. 21e), it follows that (Eqn 3 being a reasonable representation of reality and other things being equal)

$$D \propto M^{-1/3} \tag{4}$$

The reasonableness of Eqn 3 can be supported in at least three ways. First, empirical values of Stokes radius derived from measurements of limiting ionic mobilities agree satisfactorily with predictions from molecular models when $a_{eff} \gtrsim 0{\cdot}4$ nm ($M \gtrsim 200$) (Robinson & Stokes 1959; Fig. 6·1). Second, molecular dynamics simulations of diffusion show (Ould-Kaddour & Levesque 2001) deviations in the direction reported by Robinson & Stokes (1959; Fig. 6·1) and crossover to the hydrodynamics regime ($a_{eff} \doteq a_{solute}$) when $a_{solute} \gtrsim 4a_{solvent} \doteq 0{\cdot}3$ nm (for water). Third, Eqn 3 makes fairly good predictions for globular proteins *in aqueous solution* (Tanford 1961; Tables 21,1)

Unfortunately, *in cytoplasm*, experimentally determined diffusion coefficients fall off somewhat faster than $M^{-1/3}$ for $M \gtrsim 1$ kDa, thereby 'suggesting that the cytoplasm possesses a higher-order intermolecular structure that impedes large particle diffusion' (Zucker, Goessling & Gollan 1996). That is, supramolecular structure in the cytosol appears to increase the effective viscosity of the cytosol to the motion of large particles.

## Diffusive irreversibility

Consider a sphere of radius $a$ which is centred on the origin of a spherical coordinate system and filled with a homogeneous solution in which particles of a certain type $\gamma$ have a diffusion coefficient $D$. Suppose, that at time $t = 0$, a uniform swarm of $N_0$ $\gamma$-particles is released within a sphere of radius $\alpha << a$ (also centred on the origin) and allowed to diffuse outwards. It is shown in Eqn A6 of the Appendix that the swarm spreads outward and approaches equilibrium with a characteristic time

$$\tau_{spreading} = a^2/(2\pi^2 D). \tag{5}$$

Observe that this time is independent of $\alpha$.

Consider next the converse case in which it is desired to gather up the $\gamma$-particles after they have diffused to form a uniform equilibrium density $C_0$. To this end, let an absorbing sphere of radius $\alpha << a$ be placed at the origin. It is shown in eqn A10 of the Appendix that the gathering up approaches equilibrium with a characteristic time which is much larger than that of spreading:

$$\frac{\tau_{\text{gathering}}}{\tau_{\text{spreading}}} = \frac{2\pi^2}{3}\frac{a}{\alpha} = 6.579\ldots\frac{a}{\alpha} \quad (6)$$

One reasonable conclusion which can be drawn from Eqn 6 is that it would appear markedly inefficient for the cell to transport time- or location-sensitive materials diffusively.

## Diffusion on a shell

Equation 5 for spreading time presumed that the cell interior is homogeneous fluid whereas, in most mature *plant* cells, it is mostly vacuole with a some peripheral cytoplasm; current opinion is that the fraction of protoplasm devoted to multiple varieties of vacuole (Bassham & Raikhel 2000) is seldom less than 30% and frequently as much as 90% (Taiz & Zeiger 1998; pp. 20 & 61; Staehelin & Newcomb 2000; p. 25). Hence a potentially relevant question is that of the relaxation-to-equilibrium of the probability-density of a diffusible entity in a thin spherical shell of featureless cytosol. This problem is also treated in the Appendix and shown to lead to a characteristic time roughly

$$\tau_{\text{shell}} = a^2/(2D) \quad (7)$$

## Reaction–diffusion

The mathematics of this case is discussed briefly in the Appendix where it is shown that the dimensionless parameter $\Xi = a\sqrt{\frac{\lambda}{D}}$ should be rather less than $\pi$ if the peripheral regions of the diffusing volume are not to be starved for reactants being emitted by a non-local source; here $\lambda$ ($s^{-1}$) is the reaction rate. Because the diffusion of large particles in cytoplasm is often markedly slower than in aqueous solution (Mastro & Keith 1984; Luby-Phelps 2000), effective diffusion coefficients as low as $10^{-12}$ $m^2$ $s^{-1}$ seem realistic; and this means that even reactants with survival times as long as 10 s could become scarce at subcellular dimensions. On the other hand, given the presence of moving sources of reactant, all reactant sinks might in time-average (i.e. ergodically) be adequately sourced, if (i) the cell had streaming along randomly varying trackways; or (ii) streaming were initiated locally in response to resource depletion (Reuzeau *et al*. 1997).

## Convection–diffusion

It has been forcefully pointed out by Reuzeau *et al*. (1997) that resource depletion at a sink could be ameliorated by moving the sink into less depleted regions; and further, 'Solute distribution within the bulk cytoplasm of each cell ought to be a combination of self diffusion and cyclosis.' (Tyree 1970). These ideas seem attractive intuitively but, given the non-intuitive qualities of low Reynolds number flow, could profitably be subjected to theoretical validation.

Consider a reactant-absorbing sphere of radius $a$ which remains fixed in a uniform flow having well away from the sphere a velocity $U$ ($m\ s^{-1}$). The velocity distribution around a sphere is well known (e.g. Milne-Thomson 1950; sect. 19·63); and it could in principle be used with Eqn A1 to find the steady-state flux to the sphere given $c \to C_0$ for $(r/a) >> 1$, $c = 0$ at $r = a$, and $\lambda = 0$. This spuriously simple problem has not (to the author's knowledge) been rigorously solved. However, various approximate analytic and numerical investigations have provided some understanding of the flux behaviour (e.g. Friedlander 1961; Levich 1962; Coutelieris, Burganos & Payatakes 1995; Verbrugge & Baker 1996).

First, the dimensionless group of interest is the Peclet number

$$P = \frac{Ua}{D} \quad (8)$$

Rough range estimates for its defining variables are: $10^{-6} \lesssim U \lesssim 10^{-4}$ m $s^{-1}$ (cf. Kamiya 1959); $10^{-7} \lesssim a \lesssim 10^{-5}$ m (cf. Staehelin 1997); $10^{-13} \lesssim D \lesssim 10^{-9}$ $m^2$ $s^{-1}$ (cf. Mastro & Keith 1984; Luby-Phelps 2000). This yields a huge range of possible Peclet numbers: $10^{-4} \lesssim P \lesssim 10^{+4}$ (dimensionless).

Second, given the many uncertainties of diffusive and convective modelling at the subcellular level, the approximate analytic treatment of Levich (1962; s. 14) seems quite detailed enough; it yields a flux $F$ (mol $s^{-1}$) of roughly

$$F = 4\pi D C_0 a\left[1 + \frac{2}{\pi}P^{1/3}\right] \quad (9)$$

and a normalized flux

$$F_{\text{N}}(P) = 1 + \frac{2}{\pi}P^{1/3} \quad (9')$$

This yields both the expected flux in the limit $P \to 0$ and a sublinear increase with Peclet number as $P \to \infty$. Numerically, $F_{\text{N}}(0) = 1{\cdot}00$; $F_{\text{N}}(10^{-4}) = 1{\cdot}03$; $F_{\text{N}}(10^{-2}) = 1{\cdot}14$; $F_{\text{N}}(1) = 1{\cdot}64$; $F_{\text{N}}(10^{+2}) = 3{\cdot}95$; $F_{\text{N}}(10^{+4}) = 14{\cdot}72$. Thus, as suggested by Reuzeau *et al*. (1997), streaming could move a reaction centre to a region where limiting reactants were more plentiful.

## Summary

Snippits which usefully might be retained from this brief overview of diffusion are

1 Averaged over relevant time scales and interior compartments of a cell, the concentrations of various diffusants can be usefully estimated by macroscopic diffusion theory in situations where random walk is the chief mechanism of diffusant transport.
2 As molecular weight increases beyond 1 kDa, the predictions of the Stokes–Einstein equation for the diffusion coefficient become increasingly less accurate as the effective viscosity increases progressively.
3 Diffusive irreversibility makes pole-to-pole diffusive transport of rare molecules highly inefficient.
4 Reaction-diffusion could lead to 'pockets of poverty' of high-demand reactants unless distribution is somehow evened out by cytoplasmic streaming.
5 Organelles whose needs for high molecular weight reactants are hard to meet by passive diffusion could receive a significant boost from high Peclet number cytoplasmic streaming. However, this is intracellular transport rather than symplastic transport.

## TOW: DOES SYMPLASTIC TRANSPORT INVOLVE TARGETED INTRACYTOPLASMIC TRANSFER OF MOLECULES?

Because timely point-to-point transport of messenger molecules and rare reactants should be markedly hindered by diffusive irreversibility, it would make sense for the cell to sequester such substances in special compartments where (i) their diffusive spread is appropriately channelled; or (ii) to move the compartments themselves. But do we have evidence of this *in situations relevant to symplastic transport*?

### Diffusion in two or one dimensions

The landmark paper of Kachar & Reese (1989) on streaming in the Characae demonstrated that the endoplasmic reticulum forms a dense network of anastomosing cisternae in the cytosol, that it is pulled along actin cables, and that it appears to metamorphose in form as it travels. Not long thereafter it was shown that membrane-soluble lipid tracers could spread from cell to cell and exhibited inferred endomembrane lateral diffusivities on the order of $10^{-13}$ $m^2$ $s^{-1}$ (Grabski, de Feijter & Schindler 1993); moreover, when plasmodesmatal connectivity was disrupted by plasmolysis, intercellular spread of label was suspended without stopping the spread of label within the intracellular endomembranes.

It would therefore appear that, if a molecule of interest were once to be attached to a laterally diffusible membrane protein of the endomembrane continuum, it could stochastically find its way to a reticular domain of interest, to a Golgi stack, or even to the nucleus. Moreover, a crude estimate of the stochastic wandering time of a reactant molecule within a sphere of radius a to find a reaction site of radius $\alpha$ is, from the Appendix,

$$\tau_{\text{sphere}} = \frac{a^2}{D}\frac{a}{3\alpha} \tag{10a}$$

whereas the corresponding time for two-dimensional diffusion in the planar region between two concentric circles of radii $a$ and $\alpha$ is

$$\tau_{\text{circle}} = \frac{a^2}{D}\ln\sqrt{a/\alpha} \tag{10b}$$

Unfortunately Eqns (10) are somewhat misleading because, although $D$ and $\alpha$ could be much the same, the total area of the endomembrane continuum is thought to be $\Gamma$-fold greater than that of the plasmalemma, crudely implying $\Gamma[4\pi a_{\text{sphere}}^2] \sim \pi a_{\text{circle}}^2$; the data reviewed by Luby-Phelps (2000) suggest that $1 << \Gamma \lesssim 100$. Thus,

$$\frac{\tau_{\text{sphere}}}{\tau_{\text{circle}}} \sim \frac{1}{3\Gamma}\frac{(a_{\text{sphere}}/\alpha)}{\ln\left[4\Gamma(a_{\text{sphere}}/\alpha)^2\right]} \tag{11}$$

To illustrate what this means, suppose that $\Gamma = 30$, $a_{\text{sphere}} = 10\ \mu$m and $\alpha = 1$ nm. This yields a ratio ~5. That is, it appears that the large values reported for $\Gamma$ have rather diminished the prospectively greater efficiency of diffusion in two dimensions. Hence, constraining diffusive spread to two dimensions may help some in making diffusion of specific reactants more effective, but not as much as might have been hoped.

If however, the endomembrane reticulum could create and preserve preferred orientations or trackways for diffusion, this would help greatly for then the problem could become one of diffusion down a line of length $a_{\text{line}}$ and (cf. Appendix)

$$\tau_{\text{line}} = \frac{a_{line}^{\ 2}}{D}\frac{4}{\pi^2} \tag{12}$$

If $a_{\text{line}} \sim a_{\text{circle}}$, if follows that

$$\frac{\tau_{\text{sphere}}}{\tau_{\text{line}}} \sim \frac{\pi^2}{48\Gamma}(a_{\text{sphere}}/\alpha) \tag{13}$$

The parameter values suggested above yield for this ratio ~70, which seems rather more attractive.

That is, *if key molecules, either macro- or micro-, could be targeted to highly mobile highly selective docking moieties in suitably architected endomembranes, pole-to-pole diffusive transport might prove useful to a cell.* At this time such transport is distinctly speculative even though highly selective docking is at present a subject of intense scrutiny, especially in animal systems. But despite the likely importance of such a process, it presumably could occur in the absence of cytoplasmic streaming.

### Appropriate channelling

Cantrill, Overall & Goodwin reported recently (Cantrill, Overall & Goodwin 1999) that two types of fluorescent probes micro-injected into explants from stem internodal tissue of either *Nicotiana tabacum* or *Torenia fournieri* could end up in one of two places: commonly in the cytosol and rarely within the endoplasmic reticulum. In the latter case, it appeared as if probes (even if 10-fold above the usual molecular weight exclusion limit for cell-to-cell passage through the plasmodesmal annulus) could travel freely from cell to cell, presumably via the desmotubule. However, a still more recent report (Crawford & Zambryski 2000) suggests that 27·5 kDa green fluorescent protein targeted to the lumen of the endoplasmic reticulum did not, in a different tobacco cultivar and different cell type, leave the transfected cell. It would appear that the endomembrane lumen might not be the pathway of choice for really interesting (i.e. high molecular weight or hormonal) messages and molecules. Moreover, because such intraluminal transport presumably does not require cytoplasmic streaming, this burgeoning area is beyond the scope of this paper.

### Organelle motion

It is clear that molecular motors, powered by ATP and running along actin filaments, are abundant within the cytosol. Empirically, they do from time to time result in blatantly obvious streaming. Presumptively, these motors also operate in the absence of organized streaming: after all, the cell does have to manage its supplies of vacuoles, vesicles, and

various other subcellular compartments. Moreover, occasional (as distinct from organized) motions of clustered reaction sites, either to acquire a better supply of substrate or to more effectively distribute product (cf. Reuzeau *et al*. 1997), would not necessarily be noticed during casual microscopic observation.

It is, however, definitely known: (i) that molecular motors can and do move endoplasmic reticulum in the Characeae (Kachar & Reese 1989); (ii) that endoplasmic reticulum exists in at least 16 functionally different types and that it is involved in such time- and place-sensitive activities as protein synthesis, oleosome formation, and vacuole- and mitochondrion-attachment (Staehelin 1997); (iii) that members of the myosin superfamily of actin-binding molecular motors are involved organelle movement, membrane trafficking, and signal transduction (Mermall *et al*. 1998); (iv) that an acto-myosin system mediates the movement of Golgi stacks (Nebenführ *et al*. 1999); and (v) that the actin cytoskeleton of plants supports not only a transport network but also a signalling network (Volkmann & Baluška 1999). This, coupled with the unsuitability of diffusion to carry out many of the needful intracellular transport (logistic) and signalling/control ($C^3$) tasks which confront the cell, is *prima facie* evidence that molecular motor activity (of which cytoplasmic streaming is the visible paradigm) is essential to intracellular transport and that at least some of that transport is specifically targeted!

Whether cytoskeleton-mediated targeted-transport is also important to symplastic transport in general has not been definitively established. However, considering the realities of diffusive irreversibility and the existence of an intracellular motile apparatus putatively elaborated to traffic vesicular packets of molecules in both animals and plants [Lippincott-Schwartz, Roberts & Hirsch 2000 (animal); Okamoto & Forte 2001 (animal); Hawes, Brandizzi & Andreeva 1999 (comparative); Cai *et al*. 2000 (plant); Bassham & Raikhel 2000 (plant); Aaziz, Dinant & Epel 2001; Nebenführ & Staehelin 2001 (plant)], inserting an egregiously inefficient diffusive step into polar transport within a plant cell would appear both needless and maladaptive. Furthermore, the actin-myosin cytoskeleton has been definitively shown to extend through the plasmodesmata (Overall *et al*. 2000) and if the basic equipment is already at hand, it would seem a good bet that, over evolutionary time, the Viridiplantae would have pressed it into service in the cause of better symplastic transport.

Therefore, the remainder of this review will explicitly presume that a chief role of cytoplasmic streaming in symplastic transport is to move messages and metabolites within the cytoplasm in a targeted and logistically efficient manner.

## DISCUSSION

Almost by definition, symplastic transport must involve many transits through plasmodesmata. To discuss in detail our present knowledge of the architecture and physiology of these supramolecular structures is well beyond the scope of this paper; and the reader is referred to the many recent review articles (e.g. Lucas, Ding & Van der Schoot 1993; Wolf & Lucas 1994; Patrick 1997; Jorgensen *et al*. 1998; Kragler, Lucas & Monzer 1998; Ding, Itaya & Woo 1999; Lucas 1999; Overall 1999; Oparka & Santa Cruz 2000; Oparka & Roberts 2001). However, without plasmodesmatal steps symplastic transport could not exist; and, in a very real sense, the role of cytoplasmic streaming is inseparable from the role of plasmodesmata because the two must couple efficiently if the transport of metabolites and messages is to be efficacious. Because in its normal state, the plasmodesma permits nonspecific (putatively diffusive) transit by small ($M \lesssim 750$) hydrophilic particles (cf. Oparka & Roberts 2001), this discussion will be divided into *three* sections: one to discourage belief that cytoplasmic streaming is *not* important in symplastic transport; one to touch upon the transport of small solutes; and one for everything else.

### An adjuvant of diffusion?

In this case, the cytoplasmic steps of symplastic transport are left to old-fashioned inefficient diffusion and a different *raison d'être* sought for cytoplasmic streaming. One such possibility is that it is an attempt to reap the benefits of combining convection with diffusion (cf. discussion above); and, picturesquely, it could be likened either to the migration of a herd in search of greener pastures or to a travelling merchant taking his goods to the customer. Certainly, it would reduce any tendency of enzyme systems toward reactant deprivation; and it would rather more equitably distribute rapidly depleted reaction products. Hence, at the very least, an adjuvant-of-diffusion dividend could be a useful byproduct of streaming in any cell.

Most animal cells function robustly without readily visible streaming, and consequently it might seem as if plants must derive a major benefit from streaming beyond that of spinning the cytosol into a complex skein of streamlines. But this is not necessarily so because animal cells do not generally possess large central vacuoles which tend to constrain diffusion to a thin parietal layer; and therefore they should possess a marginal advantage over plant cells in diffusive mixing, an advantage which streaming should tend to offset.

But animal cells can differ from plant cells in other ways as well. Some have systems for signalling and transport which have not been reported in plants such as the claudin proteins of tight junctions (cf. Heiskala, Peterson & Yang 2001) and dyneins (Lawrence *et al*. 2001) for motoring along microtubules. Furthermore, they lack plasmodesmata and therefore have no obvious basis for symplastic transport of macromolecules as connexons have never been shown to pass particles above 1 kDa (Kumar & Gilula 1996). However, actin and/or actin-like proteins are ubiquitous in the living cell (Usmanova *et al*. 1993; Meagher, McKinney & Kandasamy 2000; Bray 2001), whereas there appears to have been potent evolutionary pressure to develop plasmodesmata (Raven 1997) and once the plant

elaborated the machinery for long-distance symplastic transport of uncommon molecules, then the benefits of targeted transport in membrane-protected packets should have driven its adoption in other contexts as well:

1 The inefficiencies which arise from diffusive irreversibility are not to be undertaken lightly.
2 Left unprotected in the cytosol, valuable proteins could react at inappropriate places and times or even be recycled. mRNAs could meet an analogous fate.
3 Only by stringently controlling the distribution of molecular messages can a symplast domain solve its $C^3$ challenge (cf. Zambryski & Crawford 2000).

Cytoplasmic streaming (or rather its underlying molecular motor activity), which in a symplastically isolated cell might serve mostly as an accelerator of biochemical reactions, becomes in a symplastic syncytium a powerful tool in the organization of the supracellular domain.

## Small hydrophilic particles

A typical solute of this sort, even allowing for tortuosity through the cytosolic hydrogel, should experience $D \gtrsim 10^{-10}$ m$^2$ s$^{-1}$ and $a \lesssim 10^{-5}$ m; the unfavourable case of spreading in a thin layer of parietal cytoplasm then yields, by Eqn 7, a characteristic time of spreading $\lesssim 0{\cdot}5$ s. Such particles diffusing axially down the cytoplasmic annulus of a plasmodesma 0·2 $\mu$m long should have transit times $\lesssim 0{\cdot}2$ ms (cf. Eqn A14).

For many common small solutes, an intracytoplasmic spreading time of 0·5 s is probably often unimportant. For these, a reasonable rule of thumb might be that they are sensibly uniform throughout the cytosol. Following any disequilibration, cytoplasmic streaming would of course shorten the return to equilibrium. But the physiological utility of such shortening could be minor, despite the known difficulties in accounting for some symplastic transport (e.g. Bret-Harte & Silk 1994).

Nevertheless, less simple situations may exist. For example, consider a sequence of $N$ cells, each ideally mixed and having concentration $c_n(t)$ (mol m$^{-3}$) of some substance and a volume $V$ (m$^3$). Presume next that each ($n = 1,2,3, \ldots, N$) receives solute from the preceding cell at a rate $G[c_{n-1} - c_n]$, where $G$ (m$^3$ s$^{-1}$) is a diffusive conductance, and loses it at a rate $G[c_n - c_{n+1}]$; this example represents, for instance, a simplified model of symplastic phloem unloading (cf. Patrick 1997). Moreover, by invoking conservation of species, it reduces to

$$\frac{V}{G}\frac{\mathrm{d}c_n}{\mathrm{d}t} = c_{n-1} - 2c_n + c_{n+1} \qquad (14)$$

which is in fact a discrete analogue of the electrotonus problem for an unmyelinated axon and should lead to the observed stair-step distribution of symplastic transport (e.g. Fisher 2000; Fig. 15·30). Symplastic transport down a linear file has been discussed from the viewpoint of irreversible thermodynamics by Tyree (1970).

Additionally, for small solutes that serve as substrates for metabolic assemblies (enzyme reaction chains, supramolecular enzymes assemblages), diffusion could be a rate-limiting step. The kinetic efficiency of 'bucket brigade chemistry' should be such that substrates sometimes become locally depleted (cf. Reuzeau *et al*. 1997); and here adjuvant-of-diffusion contributions might prove decisive. In this case it has been suggested that metabolic assemblies associate with endomembranes when their products are required, and the local depletion of substrates they bring about may actually trigger the action of the molecular motor system to drag membranes laden with enzyme complexes through the cytosol (Reuzeau *et al*. 1997). Of course, flow patterns associated with low Reynold's number flow (cf. Taylor 1967) would prevent instant juxtaposition of substrates and enzyme complexes; but with streaming the diffusion distances could be so reduced that they would be bridged in short order. Such situations could occur in either isolated or symplastically connected cells.

A small solute whose transport may seem at variance with these pronouncements is the hormone auxin (indole-3-acetic acid, $M$ = 175 Da) whose polar transport is presently the subject of intense interest (e.g. Lomax, Muday & Rubery 1995; Muday & DeLong 2001). Its polar transport is believed to require asymmetric vesicle-trafficking of the efflux carrier candidate PIN1, which localizes in basal cell walls where it promotes auxin efflux into the cell wall space (Palme & Gälweiler 1999; Geldner *et al*. 2001). However, the cell wall space is apoplastic; and polar auxin transport is therefore more properly termed '*trans*-cellular' rather than 'symplastic' and falls outside the scope of this review.

In sum, small solutes may be distributed by diffusion alone in some instances; but, in others, their distribution to sites where they are needed may be facilitated by active translocation of the sites. However, because the examples cited seem not to involve cytoplasmic streaming ways essential to symplastic transport, they are outside the scope of this review; they do, however, suggest that cell-to-cell diffusion via the cytoplasmic annulus could be a viable method of reallocating common small hydrophilic solutes (e.g. Tyree 1970; Bostrom & Walker 1975; Lucas 1997; Blackman & Overall 2001). As Tyree put it 30 years ago, 'The plasmodesmata constitute the pathway of least resistance for the diffusion of small solutes.' (Tyree 1970).

## Everything else

All other molecules are presumed to move through the plasmodesma by facilitated trafficking (cf. Oparka & Roberts 2001); and, despite their known diffusibility, there is no evidence that selected species of small solutes can not also be moved by facilitated trafficking to control their destinations. The mechanisms of this trafficking have not yet been worked out in detail. But it is unambiguous that, under favourable circumstances, particles up to ~50 kDa *can* move; and these particles may be either proteins or nucleic acids (e.g. Wolf & Lucas 1994) or various probes of known dimensions (e.g. van Bel, Günther & van Kesteren 1999;

Schulz 1999). Of course, the plasmodesma is not our focus; but its coupling to the particles transported must be if we are ever to achieve deep understanding of the relationship of streaming (and its underlying molecular motors) to symplastic transport.

If we accept the hypothesis that cytoplasmic streaming is a visible sign of the targeted intracytoplasmic transport of selected entities, we do not thereby resolve the mysteries of symplastic transport; but we do establish a formal atmosphere in which provocative questions can be more sharply posed. Four such are given below.

### *The meso-scale problem*

Both endoplasmic reticulum (Staehelin 1997) and Golgi complex (Staehelin & Moore 1995) are known to bud off vesicles. Vesicles are of a size (tens to hundreds of nanometers) at which entities are known to have very low coefficients of passive diffusion (cf. Luby-Phelps 2000); hence, even neglecting the need for orderly routing, vesicle trafficking presumably will benefit from facilitation of vesicular motion. It makes sense therefore to imagine that, when the molecular motors of the cytoplasm are towing neither single naked macromolecules nor essential organelles through the cytosol, they are towing small membrane-bounded packets of molecules (vesicles); this at least is the reigning paradigm in animal physiology, although rigorous proof appears scarce (Sheetz *et al*. 1998). By far best way of understanding vesicle formation and trafficking is *direct real-time visualization with resolution on the few-nanometer level*. As, however, these meso-scale dimensions are well below the resolution of ordinary light microscopy, disposing of this problem will presumably require further developments within the continuously burgeoning area of biological microscopy; moreover, being able to follow a punctate fluorescent label over submicrometer distances is not quite the same as being able to follow shape changes clearly on the few-nanometer level. The meso-scale problem is by no means unique to symplastic transport in plants and bedevils also trafficking in nerve cells where it has been said that 'the current phenomenological data beg questions about the cellular mechanisms used not only to transport material but also to modulate activity in a process' (Sheetz *et al*. 1998).

### *The customs officer problem*

Imagine a customs officer at a remote border crossing (plasmodesma). He observes (cf. Aaziz *et al*. 2001) a highly elaborated rail system (cytoskeleton) which approaches his post closely on both sides, with even a few tracks (actin filaments) crossing the border; and switch engines (myosins) are visibly associated with these tracks. But mostly what he sees is the occasional heavy lorry with a wide load (macromolecule) and lots and lots of innocuous passenger cars (small diffusable solutes). Should not the customs officer suspect that more is crossing the border than meets the eye? What is the purpose of those rails? Why is there ever visible cytoplasmic streaming if something is not deliberately being trafficked? In addition to small hydrophilic solutes (cf. Blackman & Overall 2001) and the modest number of trafficked macromolecules specifically identified to date (e.g. Lee, Yoo & Lucas 2000), there may also be trafficking in small lipids (Grabski *et al*. 1993); and one wonders about signalling oligopeptides (cf. Bisseling 1999; Ding *et al*. 1999) or cell wall precursors. What more may be crossing that our customs officer has thus far failed to detect?

### *The boxcar problem*

The bar code labelling so familiar from the supermarket was first developed for a logistics problem: that of automatically sorting and routing boxcars. A plant cell which is, with molecular motors, moving either single molecules or packets of molecules along the cables in its cytoskeleton faces a similar challenge: it must, at each way-point, recognize the 'boxcar' (by destination and/or content) and then route it appropriately. But what is the packet's equivalent of a waybill, how is this read, and what is the molecular machinery of track switching? In particular, how do these mechanisms function in an environment where cytoplasmic streaming itself may be altering the connectivity of actin trackways? However, vesicles coated by the proteinaceous polymer COPII are probably implicated (Antonny & Schekman 2001); and the presence of suitable targeting sequences is doubtless important (Fulgosi & Soll 2001; Ossareh-Nazari, Gwizdek & Dargemont 2001). Similar issues arise also for neurons where multiple recognition and sorting steps are suspected and where 'very little is known about the specific associations of different motor proteins with different cargoes' (Sheetz *et al*. 1998). These are deep $C^3$ problems; and to dismiss them as mere exercises in targeting would be to overlook the great intrinsic complexity of even simple commercial bar coding practice (cf. Palmer 2001).

### *The facilitation problem*

When, presumptively, a vesicle (or a single molecule) has been towed to the mouth of a plasmodesma, a decision must be made: either (i) the contents of the packet are turned lose to find their own way through to the next cell; or (ii) their transit is somehow 'facilitated'. If the former choice is made, then (thanks to diffusion and/or reaction) the contents of the packet should mostly melt away; moreover, those which do make it to the next cell must then be corralled and repackaged, presumably with still more loss. Clearly 'facilitation' would appear to be the more efficacious way of proceeding. But what is facilitation and what are its minutiae? No one knows, and cartoons of possible models seem to be the rule (e.g. van Bel *et al*. 1999; Jackson 2000; Lee *et al*. 2000; Zambryski & Crawford 2000; Blackman & Overall 2001). To stimulate discussion, here are four abstract possibilities which might figure in some future solution of the facilitation problem:

1 Upon arriving in the neighbourhood of a plasmodesmal orifice, the packet is incorporated into a *cis*-reticular compartment from which its contents are somehow delivered via the endoplasmic reticulum to a *trans*-reticular

compartment for re-packeting and export. This is admittedly vague; but it recognizes that transit of the plasmodesma must be either via endoplasmic reticulum, cytoplasmic annulus, or plasmalemma, there being no other symplastic possibilities; and it builds upon the reports of Grabski *et al*. (1993) and Cantrill *et al*. (1999) on cell-to-cell communication via endomembranes.

2 The molecules within the packet are individually coupled to molecular motors at the *cis*-end of the plasmodesma and then are towed through the cytoplasmic annulus for repackaging at the *trans*-end. Such towing, if in fact it exists (cf. Zambryski & Crawford 2000), presumably would be mediated by the actin and myosin within the plasmodesma (cf. Overall *et al*. 2000; Blackman & Overall 2001).

3 The packet is already attached to myosin engines which simply switch onto an actin trackway of the plasmodesma and tow it through the cytoplasmic annulus as a unit. This process may be facilitated by gating of the annulus into a more distended state (Zambryski & Crawford 2000).

4 The packet is stuffed into the plasmodesmal orifice, and a transient turgor increase is somehow triggered in the *cis*-cytoplasm to ram it through hydraulically.

Any or none of these models may eventually be found to pertain. The important thing is to meditate upon possibilities (however, remote) with an eye towards devising testable models for the physical bases of facilitation.

## CONCLUSIONS

'Wir müssen wissen. Wir werden wissen.' David Hilbert*

So, in the final analysis, what *is* the *precise* role of cytoplasmic streaming in symplastic transport? Clearly, we do not know. But, to the green plant, streaming is obviously valuable because the Viridiplantae we see about us did not survive by frittering away valuable resources. Despite present uncertainties, we can at least erect some targets (informed *guesses*) at which experimentalists may shoot:

First, symplastic transport is, prospectively, of two superficially dissimilar varieties which probably should be treated separately: diffusion driven, as in the spreading of sucrose from sink phloem (cf. Patrick 1997); and facilitated, which presumptively requires the operation of molecular motors and leads upon occasion to visible streaming.

Second, for common hydrophilic particles below the normal mass exclusion limit ($M \lesssim 750$ in some cells), ordinary diffusion through cytosol and cytoplasmic annulus will probably suffice to operate the requisite symplastic transport; and, except in uncommon cases, cytoplasmic streaming will at most top-up this transport.

Third, to understand the trafficking in larger particles will require, at a minimum, substantial resolution of the four problems posed above: the 'meso-scale problem' of visualizing the real-time transport of messages and metabolites; the 'customs inspector problem' of enumerating the entities trafficked; the 'boxcar problem' of understanding deeply the routing of the messages and metabolites within the cytoplasm; and finally the 'facilitation problem' of effectively conveying these messages and metabolites from one cell to another by way of the plasmodesmata.

Finally, as the shade of David Hilbert would attest, the ability of man to pose a problem does not guarantee the solvability of that problem and certainly can not specify the time scale on which a solvable problem actually will be solved.

*'We must know. We shall know.' For a discussion of Hilbert and his famous aphorism see Reid (1970).

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## APPENDIX

### Diffusive irreversibility

The equation of convection/reaction-diffusion can, following the procedures outlined in Carslaw & Jaeger (1959; ch. 1), readily be obtained as:

$$0 = \frac{\partial c}{\partial t} - D\nabla^2 c + \mathbf{u}\cdot\mathrm{grad}c + \lambda c \tag{A1}$$

where $c$ (mol m$^{-3}$) is the concentration of an arbitrary diffusant $\gamma$, $t$ (s) is the time, $\mathbf{u}$ (m s$^{-1}$) is the local vector velocity of the fluid (e.g. cytosol) and $\lambda$ (s$^{-1}$) is the rate at which the $\gamma$-component is eliminated by chemical reactions.

For the purposes of the diffusive irreversibility with spherical symmetry, Eqn A1 reduces to:

$$0 = \frac{\partial c}{\partial t} - Dr^{-2}\frac{\partial\left\{r^2\dfrac{\partial c}{\partial r}\right\}}{\partial r} \tag{A2}$$

where $r$ (m) is the radial variable in a spherical coordinate system. Appropriate boundary and initial conditions for the problem of spreading are:

$$c \text{ bounded, } r \to 0 \text{ and all } t > 0 \tag{A3a}$$

$$\frac{\partial c}{\partial r} \to 0,\ r \to a \text{ and all } t > 0 \tag{A3b}$$

$$c(t;r) = \frac{N_0}{N_A}\frac{3}{4\pi\alpha^3},\ r \le \alpha \text{ and } t = 0 \tag{A3c}$$

$$c(t,r) = 0,\ \alpha < r \le a \text{ and } t = 0. \tag{A3d}$$

This system can be solved by separation of variables and an application of Sturm–Liouville theory (Churchill 1941; sect. 24–25) to yield the general Dirichlet series in time:

$$c(t;r) = S_0 + \sum_{n=1}^{\infty} S_n \exp(-t/\tau_n)\frac{\sin(\rho_n r/a)}{(\rho_n r/a)}, \tag{A4}$$

where the $S_n$ are constants relating to the initial conditions, the time constants $\tau_n$ are given by:

$$\tau_n = \frac{a^2}{D\rho_n{}^2}, \tag{A5a}$$

and the dimensionless eigenvalues $\rho_n$ are non-zero roots of the *characteristic equation*:

$$\rho_n = \tan\rho_n. \tag{A5b}$$

These roots are well known, have been tabulated (e.g. Carslaw & Jaeger (1959; p. 492), and form a sequence 4·493 . . . , 7·725 . . . , 10·904 . . . , 14·066 . . . whose members go asymptotically as $(n + {}^1/_2)\pi$. Clearly, the solution approaches its steady value with a characteristic time given by:

$$\tau_1 \doteq \tau_{\text{spreading}} = a^2/(2\pi^2 D). \tag{A6}$$

The converse problem of gathering up the spread diffusant can be treated much as above, only with Eqns (A3) modified to be:

$$c(t;r) = 0,\ r = \alpha \text{ and all } t > 0 \tag{A7a}$$

$$\frac{\partial c}{\partial r} = 0,\ r = a \text{ and all } t > 0 \tag{A7b}$$

$c(t;r)$ unspecified, $r \le \alpha$ and $t = 0$ (A7c)

$c(t,r) = C_0,\ \alpha < r \le a$ and $t = 0.$ (A7d)

Proceeding as above also yields a Dirichlet series in time, now without a constant term and with characteristic times given by the non-zero roots of the characteristic equation:

$$\rho_n = \frac{1}{1-\alpha/a}\arctan\rho_n \tag{A8}$$

The roots of Eqn A8 other than $\rho_1$ rapidly settle near $\rho_n \sim (n + {}^1/_2)\pi$. $\rho_1$ is such that, if $3\alpha/a << 1$,

$$\tau_1 \doteq \tau_{\text{gathering}} = \frac{a^2}{3D}\frac{a}{\alpha} = \tau_{\text{sphere}} \tag{A9}$$

where the newly defined $\tau_{\text{sphere}}$ will be used later. Thus,

$$\frac{\tau_{\text{gathering}}}{\tau_{\text{spreading}}} = \frac{2\pi^2}{3}\frac{a}{\alpha} = 6.579\ldots\frac{a}{\alpha} \tag{A10}$$

Obviously, in this fully three-dimensional case, diffusive spreading is *much* faster than diffusive in-gathering when $a/\alpha$ is large.

The homologous two-dimensional in-gathering problem is that of a circular sink on a planar surface and yields a characteristic equation in terms of Bessel functions:

$$0 = J_0\left(\frac{\alpha}{a}\rho_n\right)Y_1(\rho_n) - Y_0\left(\frac{\alpha}{a}\rho_n\right)J_1(\rho_n) \tag{A11}$$

Numerical solution of this equation for possible values of $\alpha$/a yields $\rho_1 = 0{\cdot}5 \pm 0{\cdot}2$. This yields an approximate solution for the important characteristic time:

$$\tau_{\text{circle}} = \frac{a^2}{D}\ln\sqrt{a/\alpha} \tag{A12}$$

The comparison between Eqns (A9) and (A12) reveals an enormous disparity in in-gathering times as $(a/\alpha)$ grows.

The homologous one-dimensional in-gathering problem for a line yields the characteristic equation:

$$\rho_n = (n - 1/2)\pi \tag{A13}$$

and

$$\tau_{\text{line}} = \frac{a^2}{D}\frac{4}{\pi^2}[1-\alpha/a]^2 \tag{A14}$$

For large $(a/\alpha)$, Eqn (A14) is virtually independent of $(a/\alpha)$.

And, finally, the spreading problem for a symmetrical cap of diffusant on a spherical shell so thin that radial diffusion can be neglected leads to:

$$0 = \frac{\partial c}{\partial t} - \frac{D}{a^2}\frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left[\sin\theta\frac{\partial c}{\partial\theta}\right] \tag{A15}$$

where $\theta$ is the azimuthal angle. Separation of variables then yields, for the $\nu$th characteristic value, the azimuthal characteristic function $A_\nu P_\nu(\theta) + B_\nu Q_\nu(\theta)$, where $P_\nu$ and $Q_\nu$ are Legendre functions, $A_\nu$ and $B_\nu$ are constants, and

$$\nu(\nu+1) = a^2/(\tau_\nu D), \tag{A16}$$

$\tau_\nu$ (s) being the characteristic time associated with a given $\nu$.

Subject to the constraint that function must be bounded over the closed azimuthal interval $[0,\pi]$, the characteristic function reduces (cf. Erdélyi 1953; sect. 3·9) to $A_\nu P_\nu(\theta)$ with $\nu = 0, 1, 2, \ldots$ The characteristic spreading time is that associated with the least non-zero value of $\nu$ or:

$$\tau_{\text{shell}} = a^2/(2D) \tag{A17}$$

## Reaction–diffusion

To illustrate the effect of chemical reactions on removing diffusing molecules from a cell's reactant pool, consider the solution to Eqn A1 in the steady-state spherically symmetric convection-free case with a small source around the origin. Eqn A1 becomes:

$$0 = Dr^{-2}\frac{\mathrm{d}\left\{r^2\dfrac{\mathrm{d}c}{\mathrm{d}r}\right\}}{\mathrm{d}r} - \lambda c, \tag{A18}$$

with the boundary conditions

$$c(\alpha) = C_0 \tag{A19a}$$

$$\frac{\mathrm{d}c}{\mathrm{d}r} = 0 \text{ at } r = a \tag{A19b}$$

This system is readily integrated (cf. Carslaw & Jaeger 1959; ch. IX) to yield

$$c(r) = C_0\frac{\alpha}{r}\frac{\Xi\cosh\Xi(1-[r/a]) - \sinh\Xi(1-[r/a])}{\Xi\cosh\Xi(1-[\alpha/a]) - \sinh\Xi(1-[\alpha/a])}, \tag{A20}$$

where a new dimensionless number $\Xi = a\sqrt{(\lambda/D)}$ has been introduced. Hence,

$$\frac{c(a)}{c(\alpha)} = \frac{\alpha}{a}\frac{\Xi}{\Xi\cosh\Xi(1-[\alpha/a]) - \sinh\Xi(1-[\alpha/a])}. \tag{A21}$$

Normally $\alpha/a << 1$; therefore, the degree to which the more peripheral regions of the sphere will be diffusant-deprived will also depend strongly upon $\Xi$, and diffusant scarcity will be marked for $\Xi \ge \pi$.