Random walks and cell size

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Summary

For many years, it has been believed that diffusion is the principle motive force for distributing molecules within the cell. Yet, our current information about the cell makes this improbable. Furthermore, the argument that limitations responsible for the relative constancy of cell size—which seldom varies by more than a factor of 2, whereas organisms can vary in mass by up to 10^{24}—are based on the limits of diffusion is questionable. This essay seeks to develop an alternative explanation based on transport of molecules along structural elements in the cytoplasm and nucleus. This mechanism can better account for cell size constancy, in light of modern biological knowledge of the complex microstructure of the cell, than simple diffusion. ***BioEssays*** **22:1018–1023, 2000.**

Introduction

The belief that diffusion can explain many aspects of intracellular molecule movement is no longer tenable, since classical (Fickian) diffusion theory cannot strictly apply to conditions within the cell as we currently understand them.[1–7] Yet simple diffusion is still often invoked, or frequently (often unwittingly) assumed, to explain intracellular transport of macromolecules in eukaryotic cells.[8] The extensive evidence against the diffusion theory will be discussed here and an alternative viewpoint will be presented.

Since diffusion should no longer be taken as the "natural" explanation (by default) for any otherwise unexplained transport process, many of our textbooks (e.g. Ref. 9) will need to be rewritten. In some cases, however, this will occur slowly and often reluctantly, because many authors are unable to decide what to substitute for diffusion, as there is no generally accepted alternative. In the past where diffusion was patently insufficient to account for a particular phenomenon, ad hoc supportive mechanisms were sometimes promulgated, leading to concepts such as "facilitated diffusion", which generally only served to hide our ignorance. In such cases, it was assumed that, at some later stage, researchers would elucidate the molecular mechanisms involved, since these transport systems, being clearly directed and directional, cannot be diffusional processes.

The "fluid" character of the cell internum has been re-evaluated over the last generation; the idea of the cell as a bag of aqueous solution is finally being replaced by an image of a crowded and highly ordered cytoplasm.[10,11] Even metabolic processes thought, as recently as 1970, to take place in bulk aqueous solution are now attributed to organized enzyme assemblies.[12,13] In turn, these assemblies do not just float around like submarines in the same cellular sea as de Duve's "cytonauts",[14] but are tethered and closely integrated with cytoskeletal structures to form a continuum.[15] It should be appreciated, however, that this continuum is extremely dynamic and any structure within it might have a very short time constant of existence. A plethora of mathematical models would be required to accommodate these circumstances[6,7] to a diffusion-based model.[16,17] Furthermore, one of the more obvious features of the cytoplasm of the living cell seen down a powerful modern microscope is that particles, granules and organelles within it exhibit very little "free movement". In contrast, Brownian motion becomes readily apparent within superficial blebs or the main body of the cytoplasm in injured, dying and dead cells. Presumably loss of function and death are associated, inter alia, with failure to maintain the infrastructure of cytoplasm. Once such (dynamic) structure is lost, diffusion would dominate, as it does in any completely fluid medium.

If diffusion on its own imposes severe limitations on the functioning of the living cell above a certain size,[9] we have to consider what alternative means of molecular movement are generally superimposed upon this (inadequate) background activity. We will use nucleocytoplasmic transport of mRNA and related functions as our paradigm, which will lead to a discussion on how such mechanisms might exert constraints on cell size.

Extent to which random thermal movements affect macromolecules in cells

Brownian motion of colloidal particles (and thermal motion of solute molecules) are ineluctable phenomena in all fluid media. Since the cell internum is not unreasonably regarded as a fluid medium (even the erythrocyte is 63% water, oddly a figure *less* than the water of hydration of a haemoglobin crystal), the inference is that intracellular molecules and multimacromolecular assemblies can, and undoubtedly do, diffuse. We accept it as indisputable that random thermal

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motion of molecules remains a prerequisite for the formation and dissociation of enzyme–substrate, receptor–ligand, and other specific intermolecular complexes. The very nature of the cell internum[10–13,15], however, makes it highly improbable that random thermal motion is responsible for intracellular molecular movement over distances greater than ~20 nanometres.[4,6]

The figure of 20 nm (an average cell radius of 6 microns being some 300 times this distance) previously proposed[7] as the maximum distance over which diffusion of intracellular molecules might be considered instrumental was based on the following arguments. First, the "microtrabecular lattice"[18] although an ephemeral and ill-defined entity that cannot be compared with structural elements such as microtubules, nevertheless has been identified by electron microscopy. Different methods of preparation reveal a gel-like matrix probably formed by transient protein–protein associations with half-lives in the millisecond range or higher, i.e. much longer than the time needed to cross its interstices by diffusion. The linear dimensions of this lattice average 10–20 nm.[18,19] Second, the "diffusivities" of inert hydrophilic macromolecules (e.g. dextrans) microinjected into cytoplasm are 6–8 times lower than in water.[20,21] Since Sepharose beads with 50 nm pore diameters restrict the mobilities of similar-sized hydrophilic molecules by ~50%,[22] a 6–8-fold reduction in diffusivity is consistent with a spongioform cytoplasm with a mean "pore" linear dimension of ~20 nm. Provance et al.[23] estimated the average void spaces in the microtrabecular lattice at <60 nm. Lastly, from the simplest interpretation of Fick's equation, a local diffusion velocity is given by $2D/x$, where D is the diffusivity and x is the diffusion distance. For an intracellular protein, D is typically $\sim 10^{-9}$ cm^2 s^{-1},[24] so its diffusion rate averages 200, 20 and 2 μm s^{-1} over distances of 1, 10 and 100 nm, respectively. While the first of these velocities is greater than that of cytoplasmic streaming, the third is comparable and, over distances greater than 100 nm, protein movement due to diffusion will be overwhelmed by any net vectorial flow of cytoplasmic fluid. 20 nm is thus the radius within which diffusion will still play a significant part in molecular movements, but with the important caveat that this would be only where there is *no trace of convective movement*. This figure should not be taken as an absolute value, but as a reasonable order-of-magnitude estimate.

Diffusion and constancy of cell size

The one particular quasi-constant biological observation that still appears to militate in favour of diffusion-based models has classically been seen as the narrow size range of cells,[25] which contrasts with the enormous size range in organisms themselves:

> "... the diversity of sizes in which organisms occur—roughly 10^8 fold in length or, less comprehensibly, 10^{24} fold in mass. In view of this wide range, anything biological that does not vary in size ought to strike us as noteworthy ... the cells of animals are almost all within a factor of two of being ten micrometres across ... Constancy of size ought to direct our attention to the existence of some underlying physical constraint." (Ref. 9, p.158)

This argument crucially hinges on diffusion being the underlying constraint. Thus diffusion supposedly limits the distance that informational molecules (e.g. mRNAs) can travel from the nucleus before consequent delays in, e.g., protein synthesis undermine the ability of the cell to meet its metabolic load. But this would need to be measured within the living cell. Berg[17] gives a simple account of how diffusion can be demonstrated in vitro, using procedures based on those described by Fick.[26] An approximation to the Gaussian concentration curve predicted from diffusion theory, however, can only be achieved in practice if the diffusing material is inserted as a narrow band into a stable density gradient free from the disturbances of convective flow and observed over a period extending to several weeks. In contrast, the interior of a *living* cell does not resemble a density gradient, net fluid flows occur almost unceasingly and Berg's experiment remains impossible for the living cell even with the latest technology. In addition, cellular distributions of informational macromolecules seem to be largely unrelated to fluid-dynamic considerations.

The cryodissection technique of Horowitz et al.[27] demonstrated that only about 12% of cytoplasmic proteins enter an internal gelatin reference phase inside the amphibian oocyte,[24] indicating that 88% were not free to "diffuse". On two-dimensional gels, this mobile 12% of cytoplasmic proteins failed to resemble the nuclear protein population, suggesting that cytoplasmic-to-nucleus protein movement does not involve diffusion-based mechanisms. Large incisions in the nuclear envelope in situ left the nucleocytoplasmic distribution of proteins[28] or RNAs[29] unaltered. Whereas diffusivity would vary inversely with the cube root of molecular mass (particle radius) and directly with temperature, intracellular protein movements show neither a consistent relationship with molecular mass nor the expected temperature dependence.[20,21]

In a diffusing system, a slowly migrating molecule would spend longer in each instantaneously homogeneous compartment than would a fast-moving molecule, and should therefore show a relatively greater random element in its distribution. The cytoplasm proteins identified in the above studies[24,28,29] had highly non-random distributions, implying strong binding at their in vivo locations, with scarcely no migration. This suggests that the binding of proteins in vivo is the principal determinant of their final distributions, not any "random" element during movement. Most cell proteins, and probably all macromolecules, spend far more of their lives bound than mobile (see next section). Yet "diffusion" still seems to remain the default explanation. Referring to the movement of mRNA from the nucleus, Vogel notes that:

> "Most of the information, transferred from nucleus to cytoplasm as messenger RNA, is never moved more than perhaps 5 μm. Somehow the system has evolved as if transmission lines were extremely costly, as if minimization of the distance the information involved in protein synthesis must be moved has been accorded a very high priority . . .
>
> What seems to be the case is that the amount of information involved in protein synthesis is so vast that no organism has ever devised a way to disseminate it other than by simple diffusion". (Ref. 9, p.168–170)

It is not at all clear why the high information content of a transport substrate should make alternatives to diffusion untenable. Indeed, eukaryotic cells construct transmission lines; the role of cytoskeletal elements such a microtubules in moving cellular components and even organelles is common knowledge. Axonal transport alone negates this 5 μm idea, so why 5 or so μm anyway? Messenger RNA is not "soluble" or freely mobile in the fluid phase at any stage of its journey from the nucleus to the cytoplasm (reviewed in Refs. 30–33). Indeed, there is recent evidence for dynamic, mobile structures *within* the nucleus that inter alia play an important role in export of material to the cytoplasm, though even here authors are unable to refrain from using "diffusion" in their titles (q.v. 34). At the nuclear pore complex, karyopherins (transportins—exportins and importins) are seen as "receptors" with cargo-docking properties which are involved in the precise (directional) movement of their ligands in and out of the nucleus through pore complexes.[35,36]

Thus the argument that cell size is constrained by diffusion-imposed limitations on mRNA migration is both obscure and false. However, the problem of cell size constancy has to be resolved, and its relationship to the viable range of mRNA transport distances remains intuitively reasonable. So if diffusion is not important in mRNA transport, why are mRNA transit ranges supposedly so limited, and has this indeed anything to do with the near constancy of cell size?

Messenger RNA transport

Nucleocytoplasmic transport processes have been the subject of intensive research for 20 years, with much emphasis focused on the nuclear envelope (particularly the pore complexes; Refs. 32,33,35–39), but it is now clear that cell components not structurally related to it also play crucial roles.[7,30,42,44] The cytoplasmic anchoring of potential nuclear proteins such as transcription factors[42,43] and the involvement of cytoplasmic components with the engagement of transport substrates with pore complexes[45,46] are now well-attested. The idea that mRNAs and other macromolecules are *conducted* between the two main cellular compartments by a solid-state transport system has enjoyed considerable popularity,[30–33] although it is still difficult to reconcile any particular solid-state model with the facts.[46] For example, even if a continuous fibrillar system extends from the nucleus to the cytoplasm,[47,48] the fibril types must change with location (for example, they cannot be actin microfilaments throughout), so there will be no single motortype, as in axonal transport along neurons. A more plausible description of the mRNA transport system is a "dynamic gel", an array of transient fibrils of which the transport substrate itself is a component.[7] The mRNA-protein meshwork envisaged here must assemble and disassemble continually, making and breaking links with the pore complexes, the RNA components moving centrifugally on average with successive assembly steps. This is an outline scheme rather than a model, since the energy-transducing and signal-recognition equipment of the pore complex involved in macromolecular transport has not yet been assimilated into it. Nevertheless the "dynamic gel" idea fits more comfortably than any diffusion model with the available experimental evidence and theoretical predictions.

In essence, the "dynamic gel" hypothesis relies on the fact that most intracellular macromolecules tend to stick to other macromolecules. In addition to the many proteins known to associate specifically with mRNA, non-specific RNA–protein and protein–protein interactions abound in an environment in which the macromolecules are highly concentrated. Even a very abundant protein present in 10^8 copies per cell (i.e. roughly the number of histone molecules in a mammalian diploid cell) that binds to other molecules with fairly low affinity—perhaps with dissociations constants of around 10^{-7} M, or about 10^6 molecules per cell—will be 93% bound and 7% unbound at any instant.[7] This is consistent with the experimental findings of Paine and others,[24,27–29] and suggests that all macromolecules at any instant are either loosely bound to their neighbours, or newly dissociated from them and about to reassociate, a scenario that accords with Wolosewicz and Porter's "microtrabecular lattice."[49]

Possible ways in which a "dynamic gel" functions as a transport system for macromolecules have already been suggested;[7] they include negative co-operativity in recently occupied binding sites, and the effects of vectorial fluid flow through the gel. Negative co-operativity entails a brief lag period in the restoration of the "normal" affinity of a binding site. Both this phenomenon and vectorial fluid flow would make this non-specific type of transport *directional* rather than "random". If a non-specific system of this kind, involving negative co-operativity (and perhaps superposed fluid fluxes), were coupled to a specific and vectorial transport apparatus, such as the nuclear pore complex, then its actual directionality and the substrate on which it operated would be determined by the activity of that specific apparatus.

mRNA transport and constancy of cell size

Presuming for the present argument that mRNA transport does involve a quasi-solid-state process of the type outlined here and elsewhere,[7] what are the implications regarding cell size constancy? There are two main considerations. The first is that each translationally active messenger would ultimately become anchored to microfilaments or microtubules, depending on the cell and messenger type or, in the case of secreted

proteins, to the endoplasmic reticulum.[30,31] Once again, binding at the in vivo location is the main determinant of distribution. Inactive messenger will probably bind to the first available "recognition" site that it meets on an outward journey from the nucleus. On average, therefore, total messenger abundance should decrease quasi-exponentially with increasing distance from the nuclear surface.

The second consideration is that, if the components of the "dynamic gel," including the messenger itself, were to migrate outwards together (or, alternatively, the protein components were to be fairly uniformly distributed in the cytoplasm), then the probability of an assembly event will decline with the square of the distance from the nuclear surface, assuming complex formation depends on the concentration of the components. This effect alone, discounting the site anchorings of messengers that appear to be prerequisites for translation, will make the overall rate of mRNA migration inversely proportional to the square of the distance from the pore complex. This is the same relationship predicted from diffusion theory but the transport mechanism would not necessarily have this property if the distribution of binding components were non-uniform. An inverse square relationship is therefore *not* an inevitable prediction of the proposed model, as with the diffusion-based model.

If the rate of messenger migration imposes an upper limit on cell size, this could be a cogent explanation for the remarkable constancy of cell size without recourse to the diffusion theory. Whatever the exact mathematical dependence of migration rate is on the distance from the nucleus, it is unlikely to be a simple correlation. Moreover, if the "dynamic gel/quasi solid-state" model applies to macromolecule movements in general rather than just to the export of mRNA, then it is easy to see that if cell size doubles, macromolecule transport near the cell periphery would be much slower; perhaps four times slower. Peripheral processes limited by this rate of transport would be impaired, imposing an upper limit to cell size unless some additional and more energetic mechanism of distribution is invoked.

A model that is flexible in its predictions, e.g. in regard to the mathematical relationship between transport rate and distance, is ipso facto difficult to test critically. We are aware of the need to develop the model in ways that will overcome this objection, but at least there is some useful corroborating evidence to be found in the literature. For instance, increased temperature will randomise the distribution of binding components in the cytoplasm and consequently *decrease* the transport rate of a macromolecular substrate, whereas diffusion theory would have predicted an *increase* with temperature;[29] experimentally, the rate decreases.[21]

As to the lower limit of cell size, the current view is that this is set by the need to assemble the requisite machinery for packaging, maintaining, expressing and replicating the genome. Morowitz and Tourtellotte[50] have given some thought to the problem of *minimum* cell size, and this would equally lead us to consider the situation in an organism such as *Physarum polycephalum*. Here, no discrete (cell) boundaries are found, and yet nuclei are regularly spaced within the syncytium. The "regulator" could be the distance constraint on "dynamic gel" formation, with an approximate figure of perhaps ~ 1 nucleus per 10 μm linear radius. In hepatocytes, cell size depends on the level of ploidy and/or multiple nuclei, a relationship that might lend itself to the theoretical calculations of distances, copies and transport rates.

Concluding remarks

Getzenberg[51] recently stated that:

> "The cell is a highly ordered machine in which the skeleton provides the framework on which cellular functions take place. It is now becoming apparent that what were typically considered "soluble reactions" are rare, if existent at all. The structural systems contribute more to the cell than a framework for shape, although this is an important function. Cellular shape is reflecting what a cell is, does and will be."

This is a reaffirmation of basically what Sherrington[52] and Peters[53] had said 50 years ago. In addition to this "dynamic gel" idea, we also need to remember that fluid moves through this fabric. One of us has recently discussed this issue at length, which also addresses the problem of the nature of Peters' cell "capillaries."[53] In a structural sense, it is unlikely that we will find definite (lined) entities in the way Malpighi found the capillaries that completed the vascular circulation proposed by William Harvey. More likely, they will be conduits that open and close only during vectorial flow of the fluid phase of the cytoplasm. This has many implications with regard to metabolic functioning and its control, and are also discussed elsewhere.[54,55]

In this essay, we have concentrated on the role of the matrix of the cell, upon and within which reactions occur between molecules of all sizes. In addition, it is also probably the very substance that brings reactants together in an organised, directional manner, while itself moving as a whole in a centripetal manner. There are limitations in terms of the distances over which macromolecules can be transported before the rate of delivery is too slow to the more distant parts of the cell. The distance of the periphery of a cell from the nucleus, i.e. the sizing factor, will probably be a compromise between the general metabolic rate demand (but see Ref. 55) and active transport efficiency through this dynamic gel. This ironically produces a similar constraint to that of diffusion, *but for quite different reasons*, and with different consequences (predictions) for different conditions, such as alterations in temperature.

The energy requirements of the two systems is obviously an important difference, since the cell in using complex transport systems has a much higher energy requirement than if diffusion sufficed. Added to this there is the *information* needed to achieve a thoroughly organised transportation

throughout the cell, which will also be very high (see earlier quotation; Ref. 8, pp. 168–170). With diffusion, no information would be required at all, but it is now impossible to believe that the cell could leave almost everything to chance. The organisation of the cell is far from the chaos end of the spectrum, but is probably well over half way towards perfect order that might be found in a pure crystal. We have used inadequate phrases such as "a dynamic gel" and a "quasi semi-solid state" to describe the cell internum. Finally, we acknowledge that, despite the arguments against diffusion, there is good reason to consider that, within a radius of 20 nm or so, within a cell, molecules of all sizes move around by diffusion in the aqueous phase, allowing them to interact with other molecules according to their different binding affinities. Beyond this distance, their random walks become increasingly less relevant and organised transport systems are involved.

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Addendum

During the time is article has been in the throes of gestation, three articles have been published to which we wish to draw your attention.

First, there has been a publication reporting a genetic component which seems to be able to influence organism size in *Drosophila*, and interestingly is reflected by a smaller cell size rather than the body being comprised of fewer cells,[1] this clearly adds further complexity to the cell size conundrum.

Second, Phair and Misteli[2] have reported ''high mobility'' of proteins in mammalian cell nuclei. Although the distances needed to be travelled within the nucleus are far less than across the whole cell, the nucleus is even more packed with proteins, nucleic acids and other molecules than the cytoplasm. Yet the evidence from FLIP and FRAP techniques (fluorescence loss in photobleaching, and fluorescence recovery after photobleaching) would indicate high mobility within this domain by ''simple'' diffusion.

And third, Hochachka[3] discusses at length the possible models for intracellular movement of molecules, and firmly concludes that the model on which we have based our above arguments is the preferred one, rather than presume that many (most?) interactions inside cells occur by free diffusion.

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