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Minireview

Mobility of cell surface receptors: a re-evaluation

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Abstract It has long been known from fluorescence recovery after photobleaching experiments that the mobility of most cell surface receptors is much smaller than expected for free diffusion of proteins in a fluid lipid bilayer. Single-particle tracking experiments are currently revealing the complexity of the constraints to free diffusion. Evidence has been obtained for several different processes: domain-limited diffusion, temporary confinement and anomalous diffusion. The type of motion exhibited by a given receptor will profoundly influence the rate of any functional process which requires movement in the plane of the membrane. In particular, anomalous diffusion greatly reduces the distance travelled by a receptor on a time scale of minutes.

Key words: Single-particle tracking; Fluorescence recovery after photobleaching; Anomalous diffusion; Membrane domain; Cytoskeleton; Receptor clustering

1. Introduction

The lateral movement of many membrane proteins is essential to their function. This may involve movement towards a specific site on the membrane as in receptor-mediated endocytosis or the formation of transient or long-lived associations between cell surface receptors. Restrictions on movement which constrain functionally related proteins to remain in close proximity are also likely to be important. Thus an understanding of the mobility of membrane proteins is essential for elucidating the mechanism and especially the kinetics of many membrane-associated functions.

The lateral mobility of a wide variety of membrane proteins has for many years been investigated by the method of fluorescence recovery after photobleaching (FRAP) [1,2]. Typically, a small (1–2 μm diameter) area of fluorescent-labeled receptors is photobleached by a focused laser beam. The fluorescence in the bleached area is monitored and recovers due to diffusion of unbleached molecules into the area. Recovery is almost always incomplete when measurements are performed on the plasma membrane of living cells. These experiments are conventionally interpreted by two components, a fraction which is immobile on the time scale of the experiment and a mobile fraction which nevertheless is normally characterised by a diffusion coefficient smaller than expected for unrestricted diffusion.

Recently, it has become feasible to observe the movements of individual cell surface receptors by the technique of single-particle tracking (SPT) (for reviews, see [3–5]). SPT involves attaching a small particle, typically 11–40 nm in diameter, to the protein of interest. Two types of particle have been utilised: fluorescent particles which are imaged by low-light-level fluorescence microscopy and gold particles which are imaged by differential interference contrast microscopy. The movement of individual proteins in the plasma membrane of living cells can be monitored by tracking the particle positions through a sequence of images. Provided that particles are well separated compared with the resolution of the optical microscope, the positions of the particles can be determined with high precision, so that the spatial resolution of the technique is of the order of 10–20 nm (compared with $\sim$1 μm in a FRAP experiment).

SPT measurements have now been performed with a number of receptors on different cell types. These experiments have revealed a considerable complexity in the motion of individual molecules. Methods which have been devised for analysing SPT data frequently depend on comparing movements over different time scales [6–12]: for random diffusion, $<r^2>/t$ is independent of time, where $<r^2>$ is the mean square displacement measured over a time interval t. An increase in $<r^2>/t$ with time is indicative of directed motion whereas a decrease corresponds to some form of constrained diffusion. Care must be exercised in analysing individual tracks. Saxton [7] has shown using Monte Carlo simulations that random movements produce apparently non-random behaviour with "distressingly high probability". Various statistical tests, however, make it clear that non-random movements of membrane proteins occur commonly. Such statistical tests may be applied to individual tracks if there are a sufficiently large number of data points [13] permitting classification into receptor sub-populations exhibiting random diffusion, constrained diffusion, directed motion or immobility. Alternatively, different populations on the same cell may be inferred from analysis of the experimental probability distribution of particle displacements [6,14,15].

2. Constrained diffusion

2.1. Domain-limited diffusion

Probably the simplest conceptual model for constrained diffusion is the domain model. According to this model, barriers to free diffusion divide the cell membrane into domains. Receptors undergo random diffusion within a domain with a diffusion coefficient comparable to that of free diffusion in a lipid bilayer. Long range diffusion occurs much more slowly

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Abbreviations: FRAP, fluorescence recovery after photobleaching; SPT, single-particle tracking; NRK, normal rat kidney fibroblastic cell; NCAM, neural cell adhesion molecule; GPI, glycosylphosphatidylinositol; LDL, low density lipoprotein; MHC, major histocompatibility complex

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and depends on the rate at which receptors can hop between domains. The first indication of the existence of domains was obtained from a FRAP experiment in which the 'immobile fraction' was found to increase with increasing size of the bleached spot [16]. Subsequently SPT experiments with gold-labeled transferrin receptors on NRK cells revealed trajectories which visually give a strong impression of the receptors hopping between domains of a few hundred nanometres diameter [17]. The existence of barriers which could form the walls of domains have been detected by experiments in which gold-labeled receptors are dragged across the cell surface by laser tweezers [18–20]. SPT and laser tweezer experiments with E-cadherin suggest that these molecules may be either corralled by the cytoskeleton or tethered to it [20].

2.2. Temporary confinement

Jacobson and coworkers [12,21] have analysed trajectories of both the neural cell adhesion molecule, NCAM, and the T-lymphocyte differentiation marker, Thy-1, by a temporary confinement model. In this model, molecules undergo free random diffusion interspersed with periods of confinement within regions of about 300 nm diameter. These regions could be similar to the domains proposed by Sako and Kusumi [17] but are also explicable by a variety of other mechanisms [21]. Thus the temporary confinement zones could consist of clusters of integral membrane proteins within which the mobile protein becomes entangled. In the case of the GPI-anchored receptor, Thy-1, it is suggested [22] that transient confinement zones could be glycolipid-rich regions corresponding to detergent-insoluble membrane fractions observed in biochemical experiments [23]. The evidence for lipid microdomains in cell membranes has recently been reviewed [24].

2.3. Anomalous diffusion

Alternatively, constrained diffusion may be interpreted by an anomalous diffusion model. Studies of transport in disordered systems have revealed many instances where anomalous diffusion occurs [25]. Anomalous diffusion in cell membranes may result from obstacles and traps (binding sites) with a broad distribution of binding energies and escape times [10].

Webb and collaborators have previously interpreted SPT measurements with fluorescent LDL bound to LDL receptors or via Fab to IgE receptors by an anomalous diffusion model [26–28]. Data for LDL receptors from our laboratory are also consistent with anomalous diffusion [6]. A possible problem with LDL is that in our hands, it is difficult to eliminate non-specific binding (see also [29]). This suggests that specifically bound LDL could bind weakly to other cellular components such as the extracellular matrix, which would complicate the interpretation of SPT data.

Recently, we have performed detailed studies of the mobility of MHC class I molecules on HeLa cells [30]. SPT studies were performed using R-phycoerythrin coupled to Fab derived from a monoclonal antibody to MHC class I [31]. This probe is the smallest (11×8 nm) so far used for SPT experiments on cells and exhibits negligible non-specific binding so that the risk of a perturbing effect of the particle is minimised. A further advantage of this probe is that it is monovalent since it is purified as a 1:1 complex of Fab:R-phycoerythrin. This obviates complications due to crosslinking which might occur with multivalent probes.

A disadvantage of R-phycoerythrin is that it photobleaches rather readily, thus limiting the number of images which we could obtain in an SPT experiment to about 20. In the experiments with HeLa cells, the time interval between images was varied from 4 to 60 s so data were obtained for time intervals from 4 s to 20 min. The displacements, r, over a given time interval t were plotted as a histogram and fitted to the probability distribution

$$P(r)dr = [r/2Dt][\exp(-r^2/4Dt)]dr \quad (1)$$

The above equation assumes random diffusion and yields the diffusion coefficient D. Determination of D over a range of times provides a test for different types of motion [10]. For normal diffusion, D is independent of time whereas for anomalous diffusion D (strictly $<r^2>/4t$) decreases over all times. The domain-hopping model predicts anomalous diffusion whilst molecules are constrained within a domain, followed by a crossover to normal diffusion as long-range motion becomes limited by the rate of hopping between domains. If a population of molecules undergo directed motion, the histogram develops a second peak at longer times [14].

The SPT experiments with MHC class I on HeLa cells provide strong evidence for anomalous diffusion. Fig. 1 shows plots of log D versus log t for data obtained from two experiments on different time scales. The negative slope of these plots demonstrates that diffusion is anomalous over all times covered by the experiment. As is usual for single cell experiments, there is some cell-to-cell variability in the parameters but the negative slope was consistently observed. Fitting the data to [28]

$$D = D_0 t^{\alpha-1} \quad (2)$$

gave a value of α of about 0.5 ($\alpha = 1$ for normal diffusion).

The domain-hopping and anomalous diffusion models are not necessarily incompatible. The results that we have obtained for MHC class I on HeLa cells could be explained by domains if the distribution of escape times from domains is sufficiently broad. But a range of models involving obstacles and binding sites can also account for these results [10]. Constraints to diffusion in membranes has variously been pro-

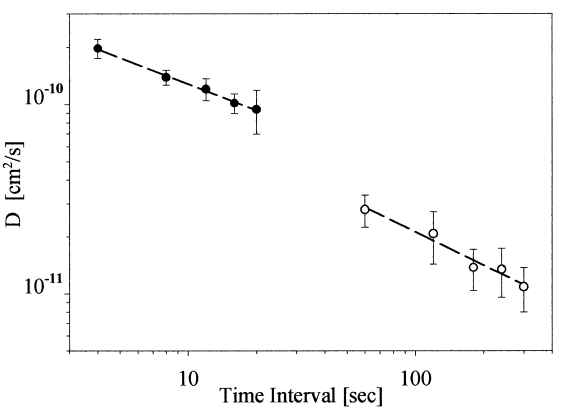

Fig. 1. Anomalous diffusion of MHC class 1 on HeLa cells. Single-particle tracks were converted into distance histograms for 1, 2, … 5 image intervals. These were analysed by Eq. 1 to give the mean diffusion coefficient and its error. Two separate experiments on different cells are shown, having image intervals of 4 s (solid symbols) and 60 s (open symbols). Linear regression on these data gives values of α of 0.54 ± 0.02 and 0.42 ± 0.07 respectively (see Eq. 2).

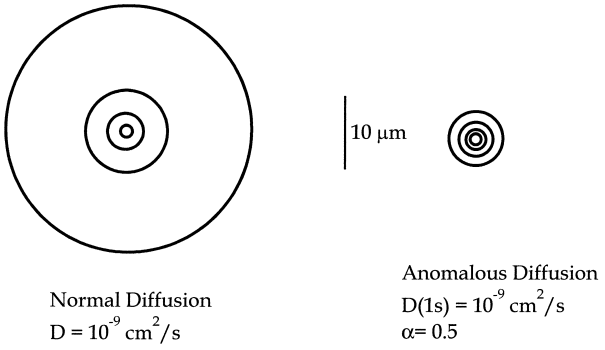

Fig. 2. Comparison of distances moved by normal and anomalous diffusion. Successive circles show the root mean square distances travelled by receptors from the centre after 1 s (innermost circle), 10s , 1 min and 10 min.

posed to arise from binding to cytoskeletal components, immobile transmembrane proteins or the extracellular matrix (reviewed in [4]). These constraints may take the form of either binding or obstruction. Of course, it is likely that there is considerable variability amongst receptors and cell types in the factors which constrain mobility and it would be unreasonable to expect a single model to be universally applicable.

3. Relationship between FRAP and SPT

The findings of SPT experiments raise questions about the interpretation of FRAP experiments. Nagle [32] proposed that anomalous diffusion might occur in cell membranes as a consequence of long-time tails in the jump rate of diffusing molecules. He analysed the effect of long tail kinetics on FRAP measurements and showed that the diffusion coefficient and immobile fraction determined by conventional means would depend on the length and time scale of the experiment. Subsequently, Feder et al. [28] analysed FRAP data for IgE receptors on rat basophilic leukaemia cells both by the conventional model of random diffusion with an immobile fraction and by a model in which all receptors undergo anomalous diffusion. They found that the two models fitted the experimental data equally well. They also carried out simulations that suggest that FRAP experiments in general are unlikely to distinguish between the two models.

A further issue is whether or not there is quantitative agreement between FRAP and SPT measurements. There is currently a paucity of evidence on this point, in part due to a number of problems in making a valid comparison. The simplest experimental system consists of lipid diffusion in model lipid membranes. Schmidt et al. [33] found fair agreement between values of D measured by SPT and FRAP for lipids labeled with a single fluorophore but Lee et al. [34] observed a two to four times lower D for SPT of lipids labeled with gold particles. The lower D appeared to be related to the multivalency of the particles.

An advantage of fluorescence SPT is that comparison with FRAP is feasible under essentially identical experimental conditions. Feder et al. [28] performed such experiments with fluorescent LDL particles attached via IgE to IgE receptors. In SPT experiments, 27% of receptors were classified as immobile. The mobile receptors mostly exhibited anomalous diffusion with mean values of $\alpha = 0.64$ and D measured over 1 s of 0.96×10^{-10} cm^2 s^{-1}. By comparison, FRAP experiments using the same probe gave $\alpha = 0.15$ and $D(1\ \mathrm{s}) = 1.4 \times 10^{-10}$ cm^2 s^{-1} when fitted to the anomalous diffusion model. A complication in these experiments was that the IgE receptors appeared to be much more mobile when FRAP experiments were performed with fluorescein-labeled IgE as the probe. This suggests that the attachment of the LDL particle significantly perturbs the receptor mobility. It will be important to determine whether this problem is confined to LDL or occurs with other particles used for SPT.

4. Functional implications

The new insights which are currently being obtained into the movement of cell surface receptors have profound implications for membrane function. A variety of functional processes require receptors to form associations or to move to specific sites such as coated pits. Theoretical analyses of such processes have generally assumed normal diffusion with diffusion coefficients derived from the mobile fraction observed in FRAP experiments. If, however, receptors undergo anomalous diffusion, then processes which occur over longer distances and time scales may be dramatically slowed down. This is illustrated by Fig. 2 which compares the distances moved for normal and anomalous diffusion. Peters [35] previously made the case that receptors are often coupled by G proteins to overcome slow diffusion in the membrane. The existence of anomalous diffusion makes this case even stronger. Yauch et al. [36] have recently proposed that altered mobility of integrins may impair cell adhesion by reducing their ability to form clusters.

On the other hand, constraints on diffusion, by whatever mechanism, could be advantageous. Receptors which are delivered to the plasma membrane by vesicle transport may not move far from the site of fusion. This could provide a means whereby functionally related receptors remain in close proximity.

5. Future studies

Further experiments need to be performed to investigate whether or not SPT and FRAP data can be incorporated within the same theoretical framework. Taken at face value, SPT experiments indicate that the random diffusion plus immobile fraction model is inappropriate for evaluating FRAP experiments. But doubts about possible effects of the particle in SPT experiments have yet to be fully resolved. One way forward will be to dispense with particles by tracking fluorescent-labeled antibodies. Schmidt et al. [33] have achieved imaging and tracking of single fluorophores though only so far in a model system. We have very recently imaged IgG bound to MHC class I on HeLa cells. The IgG was labeled at ratio of 10 fluorophores per IgG without loss of specific binding, indicating that tracking experiments on cells with fluorescent-labeled antibodies is feasible.

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