THE DISTRIBUTION OF CELL SURFACE PROTEINS ON SPREADING CELLS

Comparison of Theory with Experiment

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ABSTRACT Bretscher (1983) has shown that on uniformly spread giant HeLa cells, the receptors for low density lipoprotein (LDL) and transferrin are concentrated toward the periphery of the cells. To explain these nonuniform distributions, he proposed that on giant HeLa cells, recycling receptors return to the cell surface at the cell's leading edge. Since the distribution of coated pits on these cells is uniform, Bretscher and Thomson (1983) proposed that there is a bulk membrane flow toward the cell centers. Here we present a mathematical model that allows us to predict the distribution of cell surface proteins on a thin circular cell, when exocytosis occurs at the cell periphery and endocytosis occurs uniformly over the cell surface. We show that on such a cell, a bulk membrane flow will be generated, whose average velocity is zero at the cell center and increases linearly with the distance from the cell center. Our model predicts that proteins that aggregate in coated pits will have concentrations that are maximal at the cell periphery. We fit our theory to the data of Bretscher and Thomson (1983) on the distribution of ferritin receptors for the following cases: the receptors move by diffusion alone; they move by bulk membrane flow alone; they move by a combination of diffusion and bulk membrane flow. From our fits we show that $\tau_m > 3.5\tau_p$, where τ_m and τ_p are the lifetimes of the membrane and the ferritin receptor on the cell surface, and that $\tau_p D < 6.9 \times 10^{-7}$ cm^2, where D is the ferritin receptor diffusion coefficient. Suprisingly, we obtain the best fits to the data when we neglect membrane flow. Our model predicts that for proteins that are excluded from coated pits, the protein concentration will be Gaussian, being maximal at the cell center and decreasing with the distance from the cell center. If on giant HeLa cells a protein with such a distribution could be found, it would strongly support Bretcher's proposal that there is an inward membrane flow.

INTRODUCTION

Many receptors that mediate the internalization of extracellular ligands constantly shuttle between the surface and interior of the cell. As they return to the cell surface, they are inserted into the plasma membrane. From their insertion sites they move to coated pits where they aggregate and are internalized. Examples include the receptors for low density lipoprotein (LDL) (Basu et al., 1981; Brown et al., 1982), transferrin (Bleil and Bretscher, 1982; Harding et al., 1983; Klausner et al., 1983), asialoglycoproteins (Tanabe et al., 1960; Steer and Ashwell, 1980; Schwartz et al., 1982), and α-2-macroglobulin (Kaplan, 1980; Van Leuven et al., 1981). Bretscher (1983) studied the distribution of two of these recycling receptors, the LDL receptor and the transferrin receptor, on giant HeLa cells. He showed that on many of these cells, the distribution was nonuniform. On uniformly spread cells, transferrin and LDL receptors were concentrated toward the periphery of the cells, whereas on irregularly shaped cells, they tended to be concentrated on cell protrusions. Previously, Marcus (1962), working with giant HeLa cells infected with Newcastle disease virus, showed that newly synthesized membrane components of viral origin were added at the periphery of cells that had circular cross-sections and at protrusions of irregularly shaped cells. Similar surface distributions were observed for ferritin receptors on giant HeLa cells (Bretscher and Thomson, 1983) and transferrin receptors on spreading human epidermoid carcinoma A431 cells (Hopkins, 1985), and on normal human fibroblasts (Ekblom et al., 1983). The distribution observed for transferrin and LDL receptors on giant HeLa cells was in striking contrast to the random distribution observed for two general cell surface markers, concanavalin A (Con A) and an anti-HeLa cell antiserum (Bretscher, 1983). Also Hopkins (1985) found on A431 cells that epidermal growth factor (EGF) receptors and class 1 HLA antigens were randomly distributed. To explain the nonuniform distributions of recycling receptors on giant HeLa cells, Bretscher (1983) proposed that these receptors are

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returned to the cell surface at the cell's leading edge. The experiments of Hopkins (1985) confirm this for tranferrin receptors on A431 cells.

Bretscher and Thomson (1983) also studied the distribution of coated pits on giant HeLa cells and found them to be approximately randomly distributed over the surface. Thus, endocytosis occurs uniformly over the cell surface, but exocytosis appears to occur only at the cell periphery. Along with the recycling receptors, membrane must be returning to the cell surface at the cell's leading edge. Bretscher and Thomson (1983) concluded that if membrane is inserted at the leading edge of a giant HeLa cell and then internalized uniformly over the cell surface, there must be a bulk membrane flow away from the periphery and toward the center of the cell. To explain capping Bretscher had previously proposed that on moving cells, the endocytic cycle causes a bulk membrane flow that sweeps any large surface aggregates to the rear of the cell (Bretscher, 1976, 1982, 1984). Based on these ideas, a mathematical model of cap formation was developed that led to a simple quantitative criterion for capping (Wiegel, 1979).

Here we present a mathematical model that allows us to predict the distribution of cell surface proteins on a thin circular spreading cell, such as the giant HeLa cells in Bretscher's studies (Bretscher, 1983; Bretscher and Thomson, 1983), provided that we know the value of certain parameters. Three fundamental parameters enter the model: D, the diffusion coefficient of the protein on the cell surface; and τ_p and τ_m, the mean times the protein and the lipid membrane components spend on the cell surface before being internalized. The protein distribution is determined by these parameters through two lumped parameters: $\alpha = 1/(2D\tau_m)$ and $\gamma = 2[(\tau_m/\tau_p) - 1]$. From the distribution of a single protein, one can only determine the values of α and γ. We consider both proteins that aggregate in coated pits, such as the LDL, transferrin, and ferritin receptors, and proteins that are excluded from coated pits, such as the θ and H63 antigens on mouse fibroblast (Bretscher et al., 1980).

To illustrate that the theory can be used to analyze experiments on giant HeLa cells, we use it to fit the data of Bretscher and Thompson (1983). We also use it to predict the distribution of cell surface proteins that are excluded from coated pits. We show that the predicted distribution of these proteins, which are not endocytosed by coated pits, is nonuniform with their concentration being maximal at the center of the cell. Whether such a distribution can be readily observed on giant HeLa cells depends critically on the density and lifetime of coated pits, since it is through their internalization that an inward membrane flow is created that drives proteins toward the cell center.

The details of the mathematical model are presented in the next section. However, we have attempted to write the paper so that it can be understood without reading this section.

THE MATHEMATICAL MODEL

We consider a thin spread cell. We model its surface as a two-dimensional disc of radius R. Membrane and surface proteins are inserted into the surface at the circumference of the disc. Coated pits, which internalize membrane and surface proteins, are uniformly distributed over the disc area.

First, we determine the velocity of the lipid that is induced by the insertion of membrane at the circumference and its internalization in the interior of the disc. The continuity equation requires that anywhere on the cell

$$\frac{\partial \rho_l}{\partial t} + \nabla \cdot (\rho_l v) = S, \tag{1}$$

where ρ_l is the lipid density of the membrane, v is the flow velocity of the membrane, and S is the rate at which membrane is either inserted or removed. In the interior of the disc, membrane is removed when coated pits round up and form coated vesicles. The rate of loss of lipid membrane is proportional to the lipid density and the coated pit density ρ_p, so that

$$S = -\kappa_m \rho_l \rho_p. \tag{2}$$

The quantity $\kappa_m \rho_p$ is the rate of lipid membrane internalization by a coated pit and

$$\tau_m = 1/(\kappa_m \rho_p), \tag{3}$$

where τ_m is the mean time a lipid membrane component spends on the cell surface before being internalized by a coated pit.

In our model there is circular symmetry, with flow occurring on a plane. Eq. 1 therefore becomes

$$\frac{\partial \rho_l}{\partial t} + \frac{\rho_l}{r}\frac{\partial}{\partial r}[rv(r)] = -\frac{\rho_l}{\tau_m}. \tag{4}$$

Since the lipid density remains constant on the cell surface, $\partial \rho_l/\partial t = 0$. The solution of Eq. 4 is then

$$v(r) = -\frac{1}{2\tau_m} r. \tag{5}$$

The minus sign indicates the membrane flow is radial inward. The velocity is zero at the center of the cell and increases linearly with the distance from the center.

From Eq. 5 we can immediately derive an expression for the distribution of cell surface proteins that are excluded from coated pits. These nonrecycling proteins are driven toward the center of the cell by the flow, while diffusion tends to disperse them. In the steady state they assume a Boltzmann distribution. Since the work required to move a protein a distance dr against the flow is $fv(r)dr$, where f is the frictional coefficient of the protein, the energy associated with a protein at position r is $fr^2/(4\tau_m)$. Using the Einstein relation $D = k_B T/f$, where D is the diffusion coefficient of the protein, k_B the Boltzmann constant, and

T the absolute temperature, the Boltzmann distribution becomes

$$c(r) = c(0) \exp [-r^2/(4D\tau_m)], \tag{6}$$

where $c(r)$ and $c(0)$ are the protein concentrations a distance r from the center of the disc and at the center of the disc, respectively.

To derive an expression for $c(r)$ when the protein recycles, we start with the continuity equation. Everywhere except at $r = R$, where the protein is inserted, $c(r)$ satisfies:

$$\frac{\partial c}{\partial t} = -\nabla \cdot \mathbf{j} - \kappa_p \rho_p c, \tag{7}$$

where the flux density $\mathbf{j}$ from both diffusion and convective flow is

$$\mathbf{j} = -D\nabla c + vc. \tag{8}$$

The last term in Eq. 7 represents the loss of protein through internalization by coated pits. The quantity $\kappa_p \rho_p$ is the rate at which protein is internalized by coated pits and

$$\tau_p = 1/(\kappa_p \rho_p). \tag{9}$$

where τ_p is the mean time the protein spends on the cell surface before being internalized by a coated pit.

When we substitute Eq. 8 into Eq. 7 with v given by Eq. 5, we obtain

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial r^2} + \left(\frac{D}{r} + \frac{r}{2\tau_m}\right)\frac{\partial c}{\partial r} - \left(\frac{1}{\tau_p} - \frac{1}{\tau_m}\right)c. \tag{10}$$

We need only solve Eq. 10 in the steady state when $\partial c/\partial t = 0$. For a fully spread cell, the outer radius is constant and a true steady state is established. For a spreading cell, the microscopic motions of the cell surface proteins are much more rapid than the macroscopic growth of the cell radius, and therefore we can treat R as a constant in calculating $c(r)$.

We will assume that τ_p is constant over the cell surface. If τ_p depends on the membrane flow velocity near a coated pit then, since the velocity is a function of position, τ_p will become a function of position. If the interaction between a receptor and a coated pit is reaction limited, then τ_p will be a constant. However, if the interaction is limited by the transport of the receptor to the coated pit, then τ_p will become a function of the velocity (Goldstein et al., 1988). In that case we take τ_p in Eq. 10 to be the mean lifetime of the protein averaged over the cell surface.

It is useful to define the following parameters.

$$\alpha = 1/(2D\tau_m) \tag{11a}$$

$$\beta = \frac{\tau_m - \tau_p}{\tau_m \tau_p D} \tag{11b}$$

$$\gamma = \beta/\alpha = 2[(\tau_m/\tau_p) - 1]. \tag{11c}$$

If we let $x = \sqrt{\alpha} r$, then in the steady state Eq. 10 becomes

$$\frac{d^2c}{dx^2} + \left(x + \frac{1}{x}\right)\frac{dc}{dx} - \gamma c = 0. \tag{12}$$

The solution to Eq. 12 for all values of γ can be written in the following form:

$$c(x) = c(0)\left[1 + \frac{\gamma}{2^2}x^2 + \frac{\gamma(\gamma-2)}{2^2 4^2}x^4 + \frac{\gamma(\gamma-2)(\gamma-4)}{2^2 4^2 6^2}x^6 + \ldots\right]. \tag{13}$$

In the Appendix we discuss other forms in which Eq. 13 can be written.

Eq. 13 was derived for a homogeneous population of proteins characterized by a single diffusion coefficient D and single lifetime τ_p. In the Appendix we generalize the model to include an immobile fraction. For this more general model, we show that it is unnecessary to explicitly introduce an immobile fraction when fitting data on the distribution of cell surface proteins. Although the interpretation of the parameters change, Eq. 13 is still valid.

It is useful to have expressions for the following integrals of $c(x)$:

$$\int_0^r c(r)dr = \frac{c(0)}{\alpha^{1/2}} xL(x) \tag{14a}$$

$$2\pi \int_0^r rc(r)dr = \frac{\pi c(0)}{\alpha^{1/2}} x^2 N(x), \tag{14b}$$

where

$$L(x) = 1 + \frac{\gamma}{2^2}\frac{x^2}{3} + \frac{\gamma(\gamma-2)}{2^2 4^2}\frac{x^4}{5} + \frac{\gamma(\gamma-2)(\gamma-4)}{2^2 4^2 6^2}\frac{x^6}{7} + \ldots \tag{15a}$$

$$N(x) = 1 + \frac{\gamma}{2^2}\frac{x^2}{2} + \frac{\gamma(\gamma-2)}{2^2 4^2}\frac{x^4}{3} + \frac{\gamma(\gamma-2)(\gamma-4)}{2^2 4^2 6^2}\frac{x^6}{4} + \ldots \tag{15b}$$

From the definition of γ, Eq. 11c, we see that when proteins are totally excluded from coated pits $\gamma = -2$. For this value of γ the series in brackets in Eq. 13 equals $\exp(-x^2/2)$ and Eq. 13 reduces to Eq. 6.

Since the velocity decreases to zero as r decreases to zero, there is always a region near the origin where the velocity is negligible and pure diffusion dominates. In particular, when $(x/2)^2 \ll 1$, Eq. 13 can be approximated as follows:

$$c(r) \approx c(0)\left[I_0(\sqrt{\beta} r) - \frac{\alpha r^2}{4} I_2(\sqrt{\beta} r)\right], \tag{16}$$

where I_0 and I_2 are modified Bessel functions. The leading

term in Eq. 13 is the exact solution to Eq. 10 when $v = 0$, or equivalently when $\tau_m = \infty$.

In the absence of diffusion ($D = 0$), the solution to Eq. 10 is

$$c(r) = c(R)\,(r/R)^{\gamma}, \tag{17}$$

where $c(R)$ is the concentration at the circumference of the cell. Eq. 17 is a good approximation to the solution to Eq. 12 when $(x/2)^2 \gg 1$.

The constant $c(0)$ in Eq. 13 is determined by the outer boundary condition. We assume proteins are inserted into the plasma membrane at the circumference ($r = R$) at a constant rate S, where S has units of proteins/(cm-s), and a fraction ϕ of these move to the top of the cell. Then from Eq. 8,

$$-D\frac{\partial c}{\partial r}\Big|_{r=R} + v(R)c(R) = \phi S, \tag{18}$$

and this equation can be used to determine $c(0)$. If the top and bottom of the cell were in identical environments, half of the newly inserted receptors would move to the top of the cell and half to the bottom, i.e, $\phi = 0.5$. At the other extreme, if the cell were anchored to a surface so tightly that no proteins could move to the attached surface, then $\phi = 1.0$. In general, $0.5 \le \phi \le 1.0$. In the experiments of Bretscher and Thomson (1983), the cells are substrate-attached and we expect ϕ to be much closer to 1 than 0.5. When we analyze the data of Bretscher and Thomson (1983), we will only be concerned with relative concentrations and will not need to know $c(0)$. Thus, in fitting data the parameters S and ϕ will not enter.

RESULTS

We have modeled a giant HeLa cell as a thin flat disc of radius R. (This means our theory applies only to cells that are approximately radially symmetric.) Proteins and membrane are inserted at the circumference and internalized uniformly over the surface of the disc. The insertion and internalization of membrane sets up a steady-state membrane flow. For the geometry we have assumed, the average velocity of this membrane flow a distance r from the center of the cell, $v(r)$, is radially inward and has a magnitude equal to $r/(2\tau_m)$ (see Eq. 5). Since the flow velocity increases with r, for large enough cells there exists a distance beyond which the motion of the proteins is dominated by the membrane flow. Also, since the flow velocity goes to zero as r goes to zero, near the center of the cell membrane flow will be negligible and the motion of the proteins will be dominated by diffusion. In a time t on a two-dimensional surface, a particle with a diffusion coefficient D moves on average a distance r, where $r^2 = 4Dt$. Differentiating this expression, we see that the average diffusional velocity in the radial direction $v_D(r) = 2D/r$. Therefore, the ratio of the flow to the diffusional velocity $v(r)/v_D(r) = \alpha r^2/2$, where $\alpha = 1/(2D\tau_m)$. This suggests that we define the following characteristic distance:

$$r^* = (2/\alpha)^{1/2} = (4D\tau_m)^{1/2}, \tag{19}$$

where r^* is the radial distance from the cell center at which the membrane flow velocity equals the diffusional velocity. When $1 \ll \alpha r^2/2$, i.e., $r^* \ll r$, flow will dominate, whereas when $1 \gg \alpha r^2/2$, i.e., $r^* \gg r$, diffusion will dominate the motion of proteins. For a protein with $D = 5 \times 10^{-10}$ cm^2/s on a giant HeLa cell of radius 75 μm, these inequalities show that membrane flow will dominate diffusion at the cell periphery if $\tau_m \ll 7.8$ h. If the cell were smaller with a radius of 50 μm, then membrane flow would be important if $\tau_m \ll 3.5$ h.

To see how big a cell must be before membrane flow becomes important, we need to know the value of α, or equivalently the values of D and τ_m. For mobile proteins on cell surfaces, D ranges from $\sim 10^{-9}$ to 10^{-11} cm^2/s. To estimate τ_m, we note that $1/\tau_m$ is the rate at which membrane is internalized. Every time a coated pit is transformed into a coated vesicle, an amount of surface area equal to $4\pi a_v^2$ is internalized, where a_v is the average radius of a coated vesicle. If λ is the rate at which coated pits are internalized ($1/\lambda$ is the lifetime of a coated pit), and ρ_p is the surface density of coated pits, then

$$1/\tau_m = 4\pi a_v^2 \rho_p \lambda. \tag{20}$$

If we let $A = \pi a^2 \rho_p$, where a is the radius of a coated pit, then Eq. 20 becomes

$$\tau_m = a^2/(4a_v^2 A\lambda). \tag{21}$$

The quantity A is the apparent fraction of the surface area covered by coated pits. (By apparent we mean that the curvature of the coated pit is not taken into account.) On giant HeLa cells, Bretscher and Thomson (1983) estimated that $A \sim 0.03$ and the lifetime of a coated pit ~ 1 min, or equivalently, that $\lambda \sim 1$ min^{-1}. The radii of coated vesicles tend to be smaller than coated pits. If we take $a/a_v \sim 2$, then for these parameter values $\tau_m = 33$ min. For a protein with $D = 5 \times 10^{-10}$ cm^2/s and this value of τ_m, $r^* = 20$ μm. Thus, if these are reasonable estimates of the parameter values, on a giant HeLa cell with a typical radius $R = 75$ μm, flow will dominate diffusion near the periphery of the cell since $R \gg r^*$. However, if $D = 5 \times 10^{-9}$ cm^2/s, then $r^* = 63$ μm and flow will be much less important. Probably the best estimates of A and λ are for human fibroblasts. For these cells $A \sim 0.01$ and $\lambda \sim 0.2$ min^{-1} (for a discussion of these parameter values see Wofsy and Goldstein, 1984), which gives a $\tau_m = 500$ min. Then for $D = 5 \times 10^{-10}$ cm^2/s, $r^* = 77$ μm and diffusion will dominate membrane flow over the entire cell surface. However, if we take $D = 4.5 \times 10^{-11}$ cm^2/s, the measured value at 28°C of the LDL receptor diffusion coefficient on JD cells, a mutant human fibroblast cell line, then $r^* = 23$ μm and flow would be important near the periphery of a large enough cell.

Eq. 21 assumes that membrane is internalized only by coated pits. If it is internalized by pinocytosis as well, then Eq. 21 overestimates τ_m. If pinocytosis occurs uniformly over the cell surface, then all the results we have derived remain unchanged, except Eqs. 20 and 21. To estimate τ_m, one must add to the right side of Eq. 20 the rate at which membrane is pinocytosed.

In the previous section we derived an expression, Eq. 13, for $c(r)$, the protein concentration a distance r from the center of the cell, and showed that it depended on two lumped parameters, α and γ. Since $\gamma = 2(\tau_m/\tau_p - 1)$, when coated pits trap the protein so that its concentration is higher in coated pits than out, $\gamma > 0$; when coated pits exclude the protein so that its concentration is lower in coated pits than out, $\gamma < 0$; and when coated pits neither trap nor exclude the protein so that its concentration is the same in and out of coated pits, $\gamma = 0$. In Fig. 1 we illustrate the following general results: when $\gamma > 0$, the protein concentration is a maximum at the periphery and decreases continuously as r decreases; when $\gamma < 0$, the protein concentration is a maximum at the center of the cell and decreases continuously as r increases; and when $\gamma = 0$, the protein concentration is constant for all r.

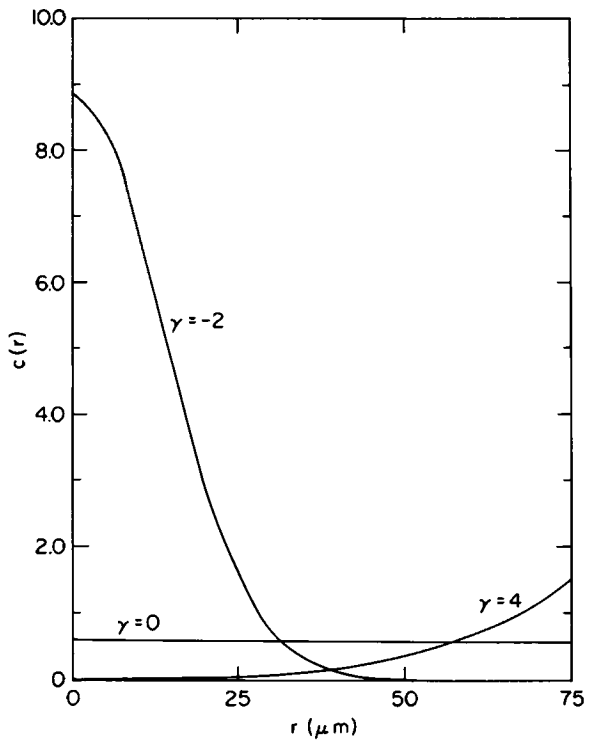

FIGURE 1 Theoretical prediction of the protein concentration a distance r from the cell center, $c(r)$, for three values of the parameter $\gamma = 2[\tau_m/\tau_p - 1]$. $\gamma = -2$ corresponds to the protein being totally excluded from coated pits; $\gamma = 0$ corresponds to the protein having the same concentration in and out of coated pits; and $\gamma = 4$ corresponds to the protein aggregating in coated pits. The total number of cell surface proteins was kept the same for all three cases. We took the cell radius $R = 75$ μm and $\alpha = 5.6 \times 10^{-3}$ μm^{-2}, which corresponds, for example, to $\tau_m = 30$ min and $D = 5 \times 10^{-10}$ cm^2/s.

When proteins are totally excluded from coated pits ($\gamma = -2$), $c(r)$ takes on the simple exponential form given by Eq. 6. Then $r_{1/2}$, the distance from the center of the cell at which the concentration drops to half its value, is given by

$$r_{1/2} = (1n2)^{1/2} r^*, \tag{22}$$

where r^* is given by Eq. 19. For $\tau_m = 33$ min we estimated that for a protein with $D = 5 \times 10^{-10}$ cm^2/s, $r^* \sim 20$ μm. For this value of r^*, $r_{1/2} = 17$ μm. However, for the same protein if $\tau_m = 500$ min, then $r_{1/2} = 64$ μm. For proteins excluded from coated pits on giant HeLa cells, the inward membrane flow will cause their cell surface distributions to be nonuniform and skewed toward the center of the cell, as illustrated in Fig. 2. Whether the internalization of coated pits can generate a large enough membrane flow for this to be readily observed is an open question. The most favorable case for observing such an asymmetry in the protein distribution is illustrated in Fig. 2. If τ_m is larger, or R smaller, or the protein is only partially excluded from coated pits ($-2 < \gamma < 0$), the asymmetry will be less pronounced.

When proteins are trapped in coated pits ($\gamma > 0$), $c(r)$ is maximum at the periphery. Flow and diffusion will move the proteins toward the center of the cell, but because the proteins aggregate in coated pits and are internalized by them, the protein concentration will be highest at the insertion sites along the cell periphery. In Fig. 3, for a protein that has a lifetime of 10 min on a giant HeLa cell of radius 75 μm, we have calculated the size of the region, as measured from the outer radius, where 90% of the protein resides. This is an annulus of outer radius $R = 75$ μm and inner radius $r_{0.9}$. For a uniform distribution on such a cell,

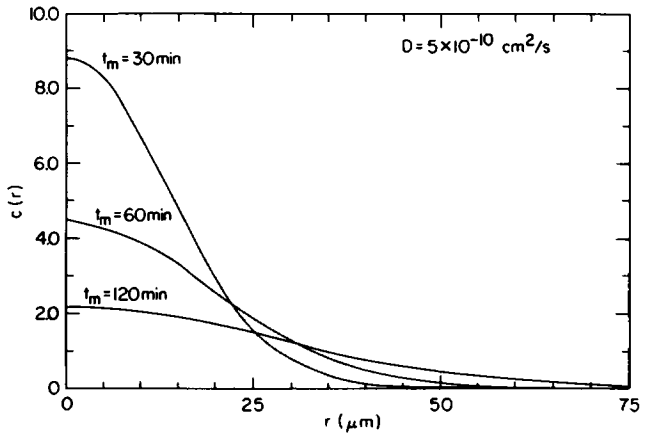

FIGURE 2 Theoretical prediction of the protein concentration a distance r from the cell center, $c(r)$, when the protein is totally excluded from coated pits ($\gamma = -2$), as a function of τ_m, the mean time the lipid membrane components spend on the cell surface before being internalized. We took the diffusion coefficient of the protein $D = 5 \times 10^{-10}$ cm^2/s and the cell radius $R = 75$ μm. As τ_m increases, the membrane flow caused by the internalization of membrane over the surface of the cell decreases.

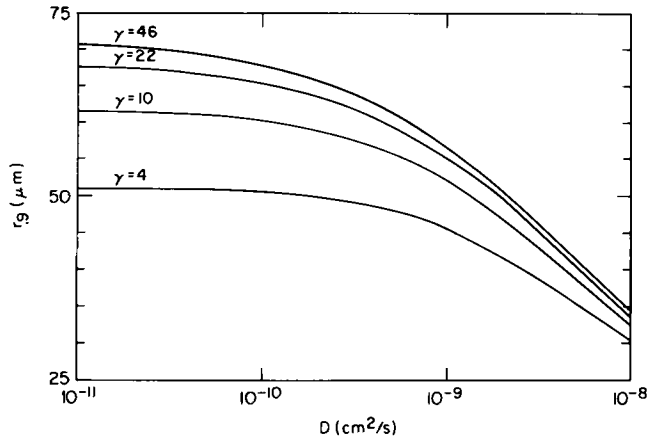

FIGURE 3 Theoretical prediction of the region where 90% of the protein resides for proteins that aggregate in coated pits ($\gamma > 0$). This region is bounded by an inner radius, $r_{0.9}$, and an outer radius, the cell radius R = 75 μm. For all curves the life-time of the protein on the cell surface, τ_p = 10 min. For this value of τ_p, the curves for γ = 4, 10, 22, 46 correspond to the lifetime of the lipid membrane components τ_m = 30, 60, 120, 240 min. As τ_m increases the net membrane flow velocity decreases. Note, for example, that for γ = 4 the curve is independent of the protein diffusion coefficient D over a large range of D values, indicating that in this parameter range membrane flow dominates diffusion as the protein transport mechanism. As the diffusion coefficient increases, all the curves converge, indicating that diffusion is now the dominant protein transport mechanism. The width of the 90% region decreases with increasing γ, i.e., $r_{0.9}$ increases, because as the inward membrane flow decreases particles travel shorter distances before they are trapped in coated pits.

$r_{0.9}$ = 24 μm. For a wide range of parameter values, we see from Fig. 3 that the model predicts that proteins that aggregate in coated pits are highly concentrated toward the cell periphery, as has been observed by Bretscher (1983) and Bretscher and Thomson (1983). The curves in Fig. 3, for γ = 4, 10, 22, 46, correspond to τ_m = 30, 60, 120, 240 min. Increasing τ_m slows the membrane flow velocity (see Eq. 21), which reduces the average distance a protein moves by convection before being internalized. This is the reason why in Fig. 3, increasing γ increases $r_{0.9}$.

When the diffusion coefficient of the protein is small ($R \gg r^*$), the motion of the protein near the periphery of the cell will be dominated by the membrane flow velocity. In the absence of diffusion, $c(r)$ takes on the simple form given by Eq. 17. From this equation it follows that when $\gamma > 0$ and $R \gg r^*$,

$$r_{1/2} \approx (0.5)^{1/\gamma} R, \tag{23}$$

$$r_{0.9} \approx (0.1)^{1/(\gamma+2)} R. \tag{24}$$

Eqs. 23 and 24 predict, for example, that when γ = 4, $r_{1/2} \approx$ 63 μm and $r_{0.9} \approx$ 51 μm. If we take diffusion into account and use Eqs. 13, 14b, and 15b to solve numerically for $r_{1/2}$ and $r_{0.9}$ we find for $D = 5 \times 10^{-10}$ cm^2/s, τ_p = 10 min, and τ_m = 30 min that $r_{1/2}$ = 60 μm and $r_{0.9}$ = 48 μm; thus, for these parameter values, the protein distributions near the periphery of the cell are close to those induced by membrane flow alone.

Bretscher and Thomson (1983) studied the distribution of ferritin receptors on normal and giant HeLa cells. They labeled giant HeLa cells that were grown on araldite substrate with horse spleen ferritin at 0°C. After these cells were washed, fixed, stained, and embedded, thin sections were cut from the middle regions of those cells that were well spread. The distribution of ferritin particles on the cells was determined by dividing each cell into five segments as shown in Fig. 4. The ferritin particles along the dorsal surface on each cell were counted. These cells had some microvilli present, but only ferritin particles along the base of the plasma membrane, including in coated pits, were recorded. Bretscher and Thomson's results are reproduced in Table I where for five cells, the number of ferritin particles per segment along with the approximate contour lengths of the segments, excluding microvilli, are given. To use our theory to fit these data, we must construct equivalent thin circular cells. These model cells should have the same surface areas as the giant HeLa cells. From the cross-section of a giant HeLa cell, one can estimate its surface area by numerical integration, if one assumes the cell is radially symmetric. Here we simply equate the diameter of the model cell with the measured contour length of the cross-section through the center of the true cell. This overestimates the cell surface area, but the errors that are introduced are small. We have also used a simple geometric model for a giant HeLa cell that underestimates the surface area. When we fit the data using these smaller dimensions, the changes in the parameter values we obtain are always less than 10% (unpublished results). In Table II we give the dimensions of the segments of the model cells and the fraction of ferritin particles in each segment.

For each cell we have three data points, the fraction of the ferritin particles in segments 1-3. We have calculated this fraction from the theory using Eq. 14a and obtained an expression that depends on the two parameters, α and γ (for details see the Appendix). To compare theory to experiment, we have fixed the value of α, and determined γ by a least-squares fit of the theory to the data. Because there are only three data points per cell, we cannot

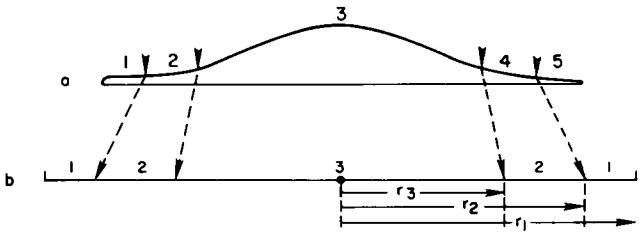

FIGURE 4 Schematic diagram of (*a*) a giant HeLa cell and (*b*) an equivalent model cell whose thickness is negligible and whose dorsal area is the same as that of the giant HeLa cell. The arrowheads indicate the borders of the segments referred to in Tables I–III. The model cell is circular with outer radius $r_1 = R$. Segment 1 is bounded by the radii r_1 and r_2, segment 2 by the radii r_2 and r_3, and segment 3 by the radius r_3.

TABLE I
DISTRIBUTION OF FERRITIN PARTICLES OVER THE DORSAL SURFACE OF GIANT HeLa CELLS AS DETERMINED BY BRETSCHER AND THOMSON (1983)

Section	1	2	3	4	5
Cell a	399(20)*	30(20)	51(62)	26(17)	183(17)
Cell b	122(11.3)	26(11.3)	104(60)	26(12.8)	307(12.8)
Cell c	479(12.0)	77(12.0)	165(73)	50(11.1)	273(11.1)
Cell d	38(7.7)	18(7.7)	225(71.5)	62(20.4)	337(20.4)
Cell e‡	349(9.2)	125(9.2)	283(63)	148(8.6)	458(8.6)

The giant HeLa cells were labeled by chilling them to 0°C and then exposing them to 5 mg/ml of horse spleen ferritin for 15 min.
*The total number of ferritin particles per segment (and the length of that segment in μm).
‡Cell e was preincubated with 100 μg/ml of cycloheximide in growth medium at 37°C for 2 h before labeling at 0°C. For details, see Bretscher and Thomson (1983).

simultaneously estimate both parameters. The relation we obtain between γ and α is shown in Fig. 5. If one of the two parameters were known, the other could be determined from this curve. For selected values of α, we give the results of these fits in Table III. From Table III we see that the theoretical fit always underestimates the amount of ferritin particles in the interior of the cell (segment 3). A small amount of nonspecific binding could explain this.

If we neglect diffusion and fit the data assuming that receptors move by membrane flow alone, the distribution will depend only on the single parameter γ. Such a fit will underestimate γ. We have done this ($\alpha = \infty$ in Table III). Averaging the three values of γ for cells, a, b, and c from Table III, we have that $\gamma > 5.0$. From the definition of γ, Eq. 11c, we find that $\tau_m > 3.5\tau_p$. That is, the lifetime of membrane lipids on the cell surface is more than 3.5 times that of the ferritin receptors.

We have also fit the data neglecting membrane flow and assuming receptors move only by diffusion. For this case the distribution depends only on the parameter $\beta(\infty) = 1/(\tau_p D)$. (From Eq. 11b we see that $\beta(\infty)$ is the value of β when membrane is not internalized so that there is no flow and $\tau_m = \infty$. This corresponds to $\alpha = 0$ in Table III.) In general, such a fit will underestimate $\beta(\infty)$ since it will overestimate the value of D in order to compensate for the membrane flow. Averaging the three values of $\beta(\infty)$ for cells a, b, and c from Table III, we have that $\beta(\infty) > 1.4 \times 10^6$ cm^{-2}, or equivalently that $\tau_p < 1/(D \times 1.4 \times 10^6$ cm^{-2}). This predicts, for example, that for a protein with a diffusion coefficient $D = 5 \times 10^{-10}$ cm^2/s, the time until the protein is internalized $\tau_p < 23$ min. The most striking result of the data fitting is that the best fits (lowest sum of squares, SS, in Table III) are obtained for pure diffusion with no membrane flow.

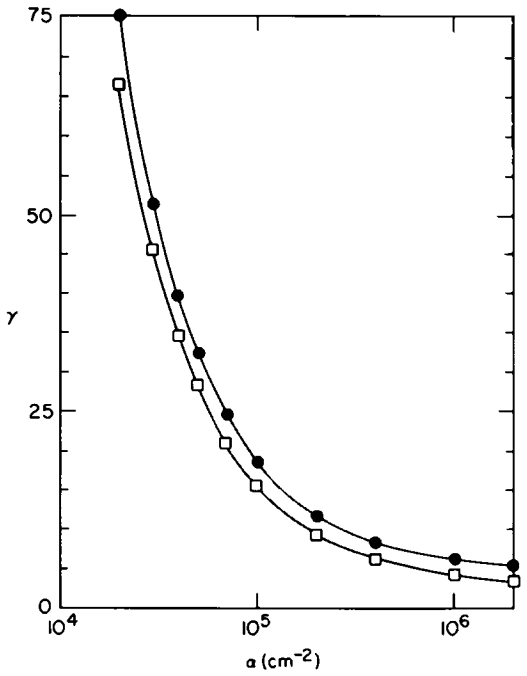

FIGURE 5 The relation between the parameters $\gamma = 2[\tau_m/\tau_p - 1]$ and $\alpha = 1/(2D\tau_m)$ obtained by fitting the data of Bretscher and Thomson (1983) in Table II. The solid circles are the average of the fits for cells a, b, and c. The open squares are the fits for cell e, which was preincubated with cycloheximide. As α approaches ∞, diffusion becomes negligible. In this limit the distribution of ferritin particles depends on the single parameter γ. As α approaches zero, γ approaches ∞ and membrane flow becomes negligible. In this limit the distribution of ferritin particles depends on the single parameter $\beta(\infty) = 1/(D\tau_p)$, which is equal to the limit of $\gamma\alpha$.

TABLE II
THE DIMENSIONS AND DISTRIBUTION OF FERRITIN PARTICLES OVER THE DORSAL SURFACE OF THE THIN, SYMMETRIC, MODEL CELLS (SEE FIG. 5) AS DETERMINED FROM THE DATA IN TABLE I

Section	1	2	3
Cell a	0.845 (68.0)*	0.081 (49.5)	0.074 (31.0)
Cell b	0.733 (54.1)	0.089 (42.1)	0.178 (30.0)
Cell c	0.720 (59.6)	0.122 (48.1)	0.158 (36.5)
Cell e	0.592 (49.3)	0.200 (40.4)	0.280 (31.5)

Cell d was not included because it was too asymmetric for the theory to apply.
*The fraction of ferritin particles per region (and the dimensions r_1, r_2, r_3, in μm; see Fig. 4).

CONCLUSIONS

We have developed a mathematical model based on the ideas of Bretscher (1983) that predicts the distribution of diffusing cell surface proteins on well spread giant HeLa cells. We model the cell as a thin circular disc. Recycling proteins and membrane return to the cell surface at insertion sites uniformly distributed along the cell circumference. Internalization by coated pits takes place uniformly over the entire cell surface. This sets up a net membrane flow inward that is maximal at the outer radius of the cell and decreases linearly to zero at the cell center. When proteins aggregate in coated pits, the model predicts

TABLE III
LEAST SQUARES FIT OF THE THEORY TO THE DATA OF BRETSCHER AND THOMSON (1983)

Cell	α	γ	β	f_1	f_2	f_3	SS
a				(0.85)	(0.08)	(0.07)	
	$\times 10^4\ cm^{-2}$		$\times 10^6\ cm^{-2}$				$\times 10^{-3}$
	0	∞	1.35	0.86	0.12	0.02	4.2
	2	72.4	1.45	0.86	0.12	0.02	4.4
	4	38.7	1.55	0.86	0.12	0.02	4.5
	20	11.9	2.38	0.86	0.12	0.02	5.2
	40	8.6	3.44	0.86	0.12	0.01	5.7
	200	6.1	12.20	0.87	0.12	0.01	6.6
	∞	5.2	∞	0.87	0.12	0.01	7.1
b				(0.73)	(0.09)	(0.18)	
	0	∞	1.46	0.72	0.20	0.08	20.6
	2	77.2	1.54	0.72	0.20	0.08	21.0
	4	40.6	1.62	0.72	0.20	0.08	21.4
	20	11.4	2.28	0.73	0.20	0.07	24.0
	40	7.9	3.16	0.74	0.20	0.06	26.1
	200	5.2	10.40	0.75	0.20	0.05	31.4
	∞	4.7	∞	0.76	0.21	0.04	34.4
c				(0.72)	(0.12)	(0.16)	
	0	∞	1.43	0.71	0.20	0.09	11.2
	2	76.0	1.52	0.71	0.20	0.09	11.6
	4	40.3	1.61	0.71	0.20	0.09	11.9
	20	11.8	2.36	0.71	0.21	0.08	13.9
	40	8.3	3.32	0.72	0.21	0.07	15.4
	200	5.7	11.40	0.73	0.22	0.06	19.0
	∞	5.1	∞	0.73	0.22	0.05	21.1
e				(0.59)	(0.20)	(0.21)	
	0	∞	1.28	0.58	0.24	0.18	2.2
	2	66.7	1.33	0.58	0.24	0.18	2.3
	4	34.8	1.39	0.58	0.24	0.18	2.5
	20	9.3	1.86	0.57	0.25	0.18	3.6
	40	6.2	2.48	0.57	0.25	0.17	4.6
	200	3.7	7.40	0.57	0.26	0.16	8.0
	∞	3.2	∞	0.57	0.27	0.15	10.3

f_1, f_2, and f_3 are the fraction of ferritin particles in segments 1–3. The values in parentheses are the experimental values obtained from Table II. In doing the least squares fit, α was held fixed and γ was varied. SS is the sum of squares for the best fit. This procedure was carried out in all cases except where diffusion was neglected ($\alpha = \infty$) or flow was neglected ($\alpha = 0$). See the Appendix for a discussion of the mathematical expressions we used to fit the data.

that their concentration will be maximal at the cell circumference and decrease as the distance from the cell center decreases. Such asymmetric distributions have been observed on giant HeLa cells for LDL, transferrin, and ferritin receptors (Bretscher, 1983; Bretscher and Thomson, 1983). When we fit the theory to Bretscher and Thomson's (1983) data on the distribution of ferritin receptors, we find that the theory underestimates the concentration of ferritin receptors near the center of the cell. Nonspecific binding or a small immobile fraction of ferritin receptors could explain this. When we neglect diffusion and fit the data assuming receptors move only by membrane flow, we find that $\tau_m > 3.5\tau_p$, where τ_m and τ_p are the lifetimes of the membrane and the ferritin receptor on the cell surface. When we neglect membrane flow and fit the data assuming that receptors move only by diffusion, we find that $\tau_p D < 6.94 \times 10^{-7}\ cm^2$. Surprisingly, we obtain the best fits of the data when we neglect membrane flow. A possible explanation is that the density of coated pits is too low and their lifetimes too long to generate a membrane flow velocity that can appreciably influence the protein distribution. Since the magnitude of the inward membrane flow velocity increases linearly with the radial distance from the cell center, the effects of membrane flow on the distribution of cell surface proteins should be more pronounced on cells with larger radii. The radii of the cells we analyzed ranged from 49 to 68 μm, but we could not detect any influence of membrane flow. However, since the data we analyzed are very limited, and there is a range of parameter values where the fits are comparable to that obtained for pure diffusion, we cannot reject such membrane flow.

Our model predicts that for proteins that are totally excluded from coated pits, the protein concentration will be Gaussian (see Eq. 6), being maximal at the cell center and decreasing with the distance from the cell center. This is the result of a competition between membrane flow that drives proteins toward the cell center and diffusion that tends to disperse them. Even when proteins are only partially excluded from coated pits, our model predicts that their concentration should be maximal at the cell center. If on giant HeLa cells one could find a protein with such a distribution, it would strongly support Bretcher's proposal that there is an inward membrane flow.

In our model we treated the proteins in the membrane as an ideal solution. Ryan et al. (1987) studied the distribution of Fc receptors for immunoglobulin E on rat basophilic leukemia cells in the presence of an applied electric field and found that their distribution differed from the ideal Boltzmann distribution. They were able to fit the data if they took into account the excluded volume of all surface proteins. From their fits they found that ~ 50% of the cell surface was covered by protein. Whether the protein excluded volume is important in understanding the movement of proteins on giant HeLa cells is unknown. Now that a simple model exists for predicting protein distributions on giant HeLa cells, this and other questions about the movement and internalization of proteins on such cells can be explored.

APPENDIX

Expressions for $c(x)$

The series in brackets in Eq. 13 is a confluent hypergeometric function. As a result, Eq. 13 can be written in the form

$$c(x) = c(0)M(-\gamma/2, 1, -x^2/2). \quad \text{(A1)}$$

For a discussion of the properties of confluent hypergeometric functions see Slater (1964). When $\gamma/2 = n$, where n is an integer, the infinite series in Eq 13 becomes finite and equal to a Laguerre polynomial, i.e.,

$$M(-n,1,-x^2/2) = L_n^{(0)}(-x^2/2). \tag{A2}$$

For $\gamma < 0$, which corresponds to proteins being excluded from coated pits, Eq. 13 can be rewritten in the following form (Morse and Feshbach, 1953):

$$c(x) = c(0) \int_0^\infty y^{-(\gamma+1)} e^{-y^2/2} J_0(yx) \mathrm{d}y. \tag{A3}$$

Including an Immobile Fraction

We consider a homogeneous population of recycling receptors, any of which are capable of becoming immobile through interactions with the cytoskeleton. We let $\kappa_i\rho_i$ be the rate at which receptors are immobilized by the cytoskeleton, and $\kappa_p'\rho_p$ be the rate at which immobile receptors are internalized by coated pits. Then, if $c(r)$ is the concentration of mobile receptors, Eq. 7 becomes

$$\frac{\partial c}{\partial t} = -\nabla \cdot \mathbf{j} - (\kappa_p\rho_p + \kappa_i\rho_i)c, \tag{A4}$$

where the concentration of immobile receptors, $c_i(r)$, satisfies the equation

$$\frac{\partial c_i}{\partial t} = \kappa_i\rho_i c - \kappa_p'\rho_p c_i. \tag{A5}$$

In the steady state,

$$c_i(r) = \frac{\kappa_i\rho_i}{\kappa_p'\rho_p} c(r). \tag{A6}$$

If we assume that the sites of interaction on the cytoskeleton are in large excess, i.e., the interaction with the cytoskeleton is far from saturation, then we can treat ρ_i as a constant. Then Eq. A4 is of the same form as Eq. 7. Since $c_i(r)$ is proportional to $c(r)$, the total concentrations of receptors, $c_T(r) = c(r) + c_i(r)$, is proportional to $c(r)$ as well. This means that Eq. 13 holds for c_T, where $c(x)$ and $c(0)$ are replaced by $c_T(r)$ and $c_T(0)$ and

$$\tau_p = 1/(\kappa_p\rho_p + \kappa_i\rho_i). \tag{A7}$$

Expressions Used for Data Fitting

Bretscher and Thomson (1983) took sections through the center of giant HeLa cells. They divided these sections into segments and determined the total number of ferritin particles per segment. From these measurements we determined for each segment f_i, the fraction of ferritin particles in segment i. When the cells are taken to be symmetric there are three segments per cell so $i = 1$, 2, or 3. From Eq. 15a it follows that

$$f_i = \frac{r_i L(\sqrt{\alpha} r_i) - r_{i+1} L(\sqrt{\alpha} r_{i+1})}{RL(\sqrt{\alpha} R)} \tag{A8}$$

where $r_1 = R$, $r_4 = 0$, and $L(x)$ is given by Eq. 15a.

For the special case when there is no diffusion ($\alpha = \infty$), it follows from Eq. 17 that

$$f_i = \frac{r_i^{\gamma+1} - r_{i+1}^{\gamma+1}}{R^{\gamma+1}}. \tag{A9}$$

When there is no flow ($\alpha = 0$), it follows from Eq. 16 that

$$f_i = \frac{\int_0^{r_i} I_0(\sqrt{\beta} r)\mathrm{d}r - \int_0^{r_{i+1}} I_0(\sqrt{\beta} r)\mathrm{d}r}{\int_0^R I_0(\sqrt{\beta} r)\mathrm{d}r}. \tag{A10}$$

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