DIFFUSION-LIMITED FORWARD RATE CONSTANTS IN TWO DIMENSIONS

Application to the Trapping of Cell Surface Receptors by Coated Pits

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ABSTRACT A variety of receptors are known to aggregate in specialized cell surface structures called coated pits, prior to being internalized when the coated pits close off. At 37°C on human fibroblasts, as well as on other cell types, a recycling process maintains a constant number of coated pits on the cell surface. In this paper, we explore implications for receptor aggregation and internalization of the two types of recycling models that have been proposed for the maintenance of the coated pit concentration. In one model, coated pits alternate between accessible and inaccessible states at fixed locations on the cell surface, while in the other model, coated pits recycle to random locations on the cell surface. We consider receptors that are randomly inserted in the membrane, move by pure diffusion with diffusion coefficient D, and are instantly and irreversibly trapped when they reach a coated pit boundary (the diffusion limit). For such receptors, we calculate for each of the two models: the mean time τ to reach a coated pit, the forward rate constant k_+ for the interaction of a receptor with a coated pit, and the fraction ϕ of receptors aggregated in coated pits. We show that for the parameters that characterize coated pits on human fibroblasts, the way in which coated pits return to the surface has a negligible effect on the values of τ, k_+, and ϕ for mobile receptors, $D \geq 1.0 \times 10^{-11}$ cm^2/s, but has a substantial effect for "immobile" receptors, $D \ll 1 \times 10^{-11}$ cm^2/s. We present numerical examples to show that it may be possible to distinguish between these models if one can monitor slowly diffusing receptors ($D < 1 \times 10^{-11}$ cm^2/s) on cells whose coated pits have relatively short lifetimes ($\leq$1 min). Finally, we show that for the low-density lipoprotein (LDL) receptor on human fibroblasts ($D = 4.5 \times 10^{-11}$ cm^2/s), the predicted and observed values of k_+ and ϕ are in close agreement. Therefore, even for the slowly diffusing LDL receptor, unaided diffusion as the transport mechanism of receptors to coated pits is consistent with measured rates of LDL internalization.

INTRODUCTION

Receptor-mediated endocytosis is the process by which normal cells take biological molecules selectively from the external environment and transport them across the plasma membrane through the use of specialized structures called coated pits (1). It has been shown that receptors for a variety of ligands aggregate in coated pits prior to internalization. It appears that some receptors require ligand binding before they can aggregate in coated pits (2, 3), while others are found to cluster in coated pits in the absence of the ligand (4, 5).

In this paper, we consider ligand-independent receptor aggregation. The low-density lipoprotein (LDL) receptor is the prime example of the ligand-independent case. When human fibroblasts held at 4°C are fixed with paraformaldehyde and then exposed to LDL-ferritin, ~70% of the bound LDL-ferritin is localized in coated pits, even though coated pits cover only ~2% of the cell surface (4). Similarly, on rat hepatocytes, 60% of the surface receptors for asialoglycoprotein are clustered in coated pits (6). Recent experiments (7) raise the possibility that to a lesser extent the epidermal growth factor (EGF) receptor on human fibroblasts also aggregates in coated pits in the absence of the ligand.

At 37°C, the coated pit density remains constant. Goldstein and Brown (8) incubated human fibroblasts with ^{125}I-LDL for 30 h. They found that after an initial lag, the rate of degradation of ^{125}I-LDL was constant. (LDL is degraded after it is internalized.) Three distinct models have been proposed for the maintenance of the coated pit density at a constant level.

The simplest one is that coated pits remain on the cell surface indefinitely (9). Another possibility is that coated

pits mediate receptor-ligand internalization by a recycling process in which they round into closed coated vesicles inside the cell, pinching off from the cell surface and eventually returning to the surface at new locations (10). Finally, coated pits may, as in the previous case, alternate between periods when they are accessible for receptor aggregation and periods when they are not, but they always reappear in the same positions (9, 11).

The three models make different predictions for the rates at which diffusing receptors hit coated pits. Adam and Delbrück (12) and Berg and Purcell (13) showed how to calculate the forward rate constant for the interaction of diffusing particles with circular traps in two dimensions for the first model, i.e., when the traps are infinitely long-lived. Here we present the diffusion-limited forward rate constant calculations for the other two models in which traps disappear and reappear either at the original location (model 2) or at a new location (model 3).

The differences in the rate constants arise because of differences in the concentration distributions of receptors about coated pits for the three models. These distributions depend on whether and for what periods the coated pit site has acted as a trap in the past. When the steady state density of traps is the same for all three models, the rate at which receptors hit coated pits should be smallest for model 1, where the continuous presence of a sink (the coated pit) at a given location depletes the receptor concentration about the coated pit. For model 2, where traps reappear at the same locations they occupied previously, the hitting rate is somewhat faster because the coated pit acts as a sink only part of the time. The hitting rate should be greatest for model 3, where coated pits appear at random locations and thus initially experience a uniform distribution of receptors rather than a distribution with the fewest receptors near the pit.

The differences between the forward rate constants for the three models would be small if the diffusion coefficient of the receptor were large enough to guarantee that the receptor would be trapped rapidly by a coated pit. In particular, if $\sqrt{4DPT} \gg 1$ (where D is the lateral diffusion coefficient for the receptor, P is the coated pit density, and T is the mean lifetime of a coated pit), then we can treat the coated pits for the purpose of calculating the diffusion-limited forward rate constant as if they were infinitely long-lived targets (14). On human fibroblasts at 37°C, $P \cong 0.31/\mu m^2$ and $T \cong 5$ min; therefore, for receptors with diffusion coefficients of $\gg 3 \times 10^{-11}$ cm^2/s, this inequality is satisfied (for a discussion of the values of the parameters P and T, see reference 15). Barak and Webb (16) have measured the diffusion coefficient of mobile LDL receptors on a mutant human fibroblast cell line at 28°C and found that $D = 4.5 \times 10^{-11}$ cm^2/s. For receptors with such a diffusion coefficient, the lifetime of the coated pits cannot be ignored a priori.

Recent evidence (11, 17–19) has now shown that at 37°C coated pits are not infinitely long-lived but rather leave the surface by rounding up into coated structures. A hotly debated question is whether or not these coated structures, referred to in Willingham et al. (11) as cryptic coated pits, remain in contact with the surface through narrow necks or whether they truly pinch off to form vesicles (18–21). It is agreed, however, that even if the coated structures remain attached, they cannot trap surface receptors; the necks are functionally closed (11, 17, 19). As we have just noted, if the diffusion of the receptor is rapid enough, the coated pits can be modeled as if they did not recycle. For slowly diffusing receptors, the rate at which they are trapped will depend on the lifetime of the coated pit, on whether it recycles to a random location or its original location and, in the latter case, on the mean return time. There is no information to date on where recycling coated pits reappear, so that both of the recycling models are still possible.

THE FORWARD RATE CONSTANT

Estimate from Experiment

Before we attempt to calculate the diffusion limit of the forward rate constant for the interaction of an LDL receptor with a coated pit, we review briefly how, from various experiments, the forward rate constant was estimated by Goldstein et al. (14). They denoted the concentration of LDL receptors in coated pits by R_p and the concentration of LDL receptors out of coated pits by R, where they defined concentration as the average number of receptors per cell. They introduced the forward rate constant k_+ through the following rate equation for R_p:

$$dR_p/dt = k_+PR - k_-R_p - \lambda_1 R_p, \quad (1)$$

where P is the surface density of coated pits, k_- is the reverse rate constant for the dissociation of an LDL receptor from a coated pit, and λ_1 is the rate at which LDL receptors in coated pits are internalized. The total flux, J, of LDL receptors into coated pits is

$$J_+ = k_+PR. \quad (2)$$

In the steady state, Eq. 1 can be solved to yield

$$k_+ = \frac{(k_- + \lambda_1)}{P}(R_p/R). \quad (3)$$

All the parameters on the right side of Eq. 3 except k_- have been determined experimentally. Goldstein et al. (14) therefore used Eq. 3 to obtain the following lower bound:

$$k_+ \geq (\lambda_1/P)(R_p/R). \quad (4)$$

For human fibroblasts, the estimated values for the parameters in Eq. 4 are (15): $\lambda_1 = 0.19 \pm 0.05$ min^{-1}, $P = 0.31 \pm 0.09/\mu m^2$, and $(R_p/R) = 2.2 \pm 0.8$. Thus, on human fibroblasts, for LDL receptors interacting with coated pits,

the forward rate constant satisfies

$$k_+ \geqq 2.3 \pm 1.6 \times 10^{-10}\ \mathrm{cm^2/s}. \quad (5)$$

We now turn to calculating k_+ for receptors that move by pure diffusion on cell surfaces, for the various possible types of coated pit behavior.

THEORY

Geometry of the Models

Under experimental conditions where all coated pits are on the cell surface, they take up only 2% of the surface area. Thus, we are dealing with a dilute system of traps. In modeling this system, we will follow Adam and Delbrück (12) and Berg and Purcell (13) and consider a single coated pit (or coated pit location) of radius a, with particles diffusing about it in an annulus of outer radius b, which assigns to the coated pit its share of the cell surface. For the case they considered, where traps are infinitely long lived, the outer radius b can be found from the relation

$$P = 1/\pi b^2, \quad (6)$$

where P is the observed coated pit density on the cell surface at 37°C. They took the inner boundary of the annulus, $r = a$, to be absorbing, and the outer boundary, $r = b$, to be reflecting. The reflecting boundary condition can be thought of in two ways, as an appropriate simplification of the true many-trap problem in which the ligand can diffuse away from one trap and be absorbed by another. First, this condition amounts to saying that on the boundary of a disk of radius b about one trap, just as many particles are moving across the boundary toward the trap as away from it. Another way to look at the reflecting boundary condition is that a particle crossing the boundary away from a trap sees the same picture on the other side, that is, another trap centered b units away. The mean time to diffuse to the second trap would be the same as the mean time to diffuse to the first trap, if the particle were simply reflected back into the original disk.

In the case where coated pits are assumed to recycle to random locations on the cell surface, Eq. 6 gives the appropriate relation between the coated pit density and the outer radius of the annulus (14). The boundary conditions are also unchanged.

When we develop the intermediate model, in which coated pits alternate between accessible and inaccessible states at fixed locations on the cell surface, we will consider receptors diffusing in regions surrounding these locations. In this case, the density of coated pit sites is greater than the observed density P of accessible coated pits, and it determines a reduced outer radius b_r of the annulus in which receptors diffuse. The reflecting outer boundary condition, which is appropriate for the other two models, must also be modified, as we will see.

Calculation of Diffusion Limits for Forward Rate Constants

There are two ways to calculate diffusion limits for forward rate constants. One method involves a steady state calculation of the flux of diffusing particles into a trap, while the other involves calculating the mean time τ for a diffusing particle to hit a trap (mean capture time). The mean capture time calculation yields k_+ through the relation

$$k_+ = 1/\tau P, \quad (7)$$

where P is the trap density.

Alternatively, k_+ can be found as the flux of particles into a trap divided by the mean particle concentration $\langle C \rangle$. For a circular trap of radius a in two dimensions,

$$k_+ = \frac{2\pi D a}{\langle C \rangle} \left.\frac{dC}{dr}\right|_{r=a}, \quad (8)$$

where $C(r)$ is the particle concentration a distance r from the center of the trap and D is the two-dimensional diffusion coefficient of a particle. Eq. 8 modifies the standard formulation of the flux method in three dimensions. The classical way to calculate the diffusion limit for the forward rate constant in three dimensions is to solve the diffusion equation in the steady state,

$$\nabla^2 C = 0,$$

for $C(r)$, the concentration of diffusing particles, for $a \leqq r < \infty$. The steady state is achieved by holding the concentration constant at infinity and setting it equal to zero at the surface of the trap (22). The diffusion-limited forward rate constant is then obtained as the flux into a spherical trap, divided by C_∞, the steady state particle concentration at infinity. In two dimensions, there is no solution to the steady state diffusion equation with these boundary conditions when $C_\infty \neq 0$, and so this prescription cannot be followed. However, when we consider particles diffusing in an annulus about the trap, there is another way to achieve a steady state. Since we are interested in particles (LDL receptors) that are constantly being inserted at random locations into the cell membrane, it is natural to set up a steady state by having particles created uniformly and at a constant rate S in the annulus. In Wofsy and Goldstein (15), we showed that under this assumption, Eq. 7 gives the result obtained by Berg and Purcell (13) using the mean capture time method to calculate k_+ for infinitely long-lived traps in two dimensions.

Fixed-Location Model

We will begin the calculations for the two recycling models by considering model 2, where traps (coated pits) return to their original locations. The equivalent three-dimensional

problem has been treated by Szabo et al. (23) and we model our problem in a very similar way. We consider a circular trap of radius a that alternates between open and closed states. The time a trap remains open (i.e., the time a coated pit remains accessible on the cell surface) is assumed to have an exponential distribution with mean $1/\lambda_1$, and the time the trap stays closed (inside) is exponential with mean $1/\lambda_2$; i.e., the probability of an open trap closing or a closed trap opening over a short time interval is proportional to the length of the time interval. The constants of proportionality or rates of closing and opening are λ_1 and λ_2.

About the trap (or more precisely, about the trap location where the trap may be open or closed, present or absent), particles diffuse, with two-dimensional diffusion coefficient D, within a larger circular region of radius b_r. To find b_r in terms of the other parameters, note that we want to assign a region of area πb_r^2 to each trap location. Then, if P_T is the trap location density, b_r satisfies: $P_T = 1/\pi b_r^2$. We can also express P_T in terms of the density P of open traps. The fraction of coated pit locations occupied at any time is the same as the fraction of time any given pit spends on the cell surface; i.e.,

$$P/P_T = \frac{1/\lambda_1}{(1/\lambda_1) + (1/\lambda_2)}. \tag{9a}$$

Then,

$$b_r = [\lambda_2/(\lambda_1 + \lambda_2)\pi P]^{1/2}. \tag{9b}$$

Mean Capture Time Method. It will be convenient in the next few sections to denote b_r simply by b. Then b will be assumed to satisfy Eq. 9b.

To calculate the mean capture time τ, we consider a particle starting a distance r from the center of the trap location, $a \leqq r \leqq b$, at time $t = 0$. We define $w_1(r)$ to be the mean capture time if the trap is open at $t = 0$ and $w_2(r)$ to be the mean capture time if the trap is closed initially. Then w_1 and w_2 satisfy the following equations (see Appendix A):

$$D\nabla^2 w_1(r) - \lambda_1 w_1(r) + \lambda_1 w_2(r) = -1; \tag{10a}$$

$$D\nabla^2 w_2(r) + \lambda_2 w_1(r) - \lambda_2 w_2(r) = -1. \tag{10b}$$

Both the inner and outer boundaries present problems that do not arise in the case of infinitely long-lived traps. There are two reasonable possibilities for the behavior of diffusing particles at the boundary of a closed trap; particles may be excluded from the region or may be free to diffuse into it. We will consider the former case first and impose the inner boundary conditions:

$$w_1(a) = 0; \tag{11a}$$

$$\left.\frac{dw_2}{dr}\right|_{r=a} = 0. \tag{11b}$$

The condition on w_2 says that a closed trap acts as a reflecting boundary. Later, we will consider the case where particles are free to diffuse into a closed trap while it is in a nontrapping state.

The reflecting outer boundary condition suitable for the case of infinitely long-lived traps must also be modified in the present setting. If a particle is on the boundary $r = b$ of a trap in a random state [i.e., open with probability $\lambda_2/(\lambda_1 + \lambda_2)$ and closed with probability $\lambda_1/(\lambda_1 + \lambda_2)$], then its mean capture time $w(r)$, defined by

$$w(r) = \frac{\lambda_2}{\lambda_1 + \lambda_2} w_1(r) + \frac{\lambda_1}{\lambda_1 + \lambda_2} w_2(r), \tag{12}$$

is the same whether the particle diffuses back toward that trap or crosses the boundary away from the trap. This leads to the reflecting boundary condition:

$$\left.\frac{dw}{dr}\right|_{r=b} = 0. \tag{13a}$$

However, w_1 and w_2 cannot be expected to satisfy reflecting outer boundary conditions individually. Particles reaching the outer boundary of a region about an open trap do not see the same picture on both sides of the boundary. Rather, across the boundary they see a fraction $\lambda_2/(\lambda_1 + \lambda_2)$ of traps open and the remaining traps closed. Then we must have $w_1(b) = w(b)$ or, equivalently,

$$w_1(b) = w_2(b). \tag{13b}$$

The solution to Eqs. 10a and 10b subject to the boundary conditions 11a, 11b, 13a, and 13b is outlined in Appendix B. The mean capture time $w(r)$ for a particle starting a distance r from the center of a random trap is given by Eq. B5. Then τ, the mean capture time for a particle starting anywhere between $r = a$ and $r = b$, is:

$$\tau = \frac{1}{\pi(b^2 - a^2)} \int_a^b 2\pi r w(r) dr. \tag{14}$$

Eq. 14 assumes that particles are equally likely to start at any position in the annulus surrounding the trap. This appears to be a reasonable assumption in the case where the diffusing particle is an LDL receptor and the trap a coated pit. After being internalized, LDL receptors recycle to the cell surface rapidly (10, 24). They appear to recycle separately from recycling coated pits (the relevant observations are that at 37°C, only 60–70% of LDL receptors are in coated pits at any time, and at 4°C, 40–50% of coated pits do not contain LDL receptors [4, 25]).

Substituting into Eq. 14 the expression for $w(r)$ given by Eq. B5, we obtain:

$$\tau = \tau_\infty + \tau_a. \tag{15}$$

Here,

$$\tau_\infty = \frac{b^4}{2D(b^2 - a^2)} \ln(b/a) - \frac{3b^2 - a^2}{8D}; \tag{16}$$

$$\tau_a = \frac{\lambda_1}{\lambda_2}\frac{(b^2 - a^2)}{2D\alpha a}\left(\frac{\delta_{00}}{\sigma_{01}}\right), \quad (17)$$

where

$$\alpha = [(\lambda_1 + \lambda_2)/D]^{1/2}; \quad (18)$$

$$\sigma_{ij} = I_i(\alpha b)K_j(\alpha a) + I_j(\alpha a)K_i(\alpha b), \quad i,j = 0, 1; \quad (19a)$$

$$\delta_{ii} = I_i(\alpha b)K_i(\alpha a) - I_i(\alpha a)K_i(\alpha b), \quad i = 0, 1. \quad (19b)$$

I_0, I_1, K_0, and K_1 are the modified Bessel functions of the first and second kind (26).

Then the diffusion limit for the forward rate constant is found from Eq. 7, $k_+ = 1/\tau P$. Since the radius $b = b_r$ in the expression for τ, where the pit location density $P_T = 1/\pi b_r^2$, it is convenient to express k_+ as $(1/\tau P_T)$ (P_T/P), or

$$k_+ = \left(\frac{\pi b_r^2}{\tau_\infty + \tau_a}\right)\left(\frac{\lambda_1 + \lambda_2}{\lambda_2}\right), \quad (20)$$

where τ_∞ and τ_a are given by Eqs. 16 and 17, with $b = b_r$ given by Eq. 9b.

The first component of Eq. 15, τ_∞, is the mean capture time for a particle diffusing in the presence of an infinitely long-lived trap, first calculated by Berg and Purcell (13). In the present setting, it is the mean time until a particle first reaches the boundary of the trap whether the trap is open or closed. The second component, τ_a, is the probability that the particle finds the trap closed, multiplied by the mean capture time for a particle starting at the boundary of the trap when the trap is closed; i.e., $\tau_a = [\lambda_1/(\lambda_1 + \lambda_2)]w_2(a)$. If $\lambda_1 = 0$, which means that the trap is infinitely long-lived, then we recover the Berg-Purcell result $\tau = \tau_\infty$.

Other interesting limiting cases involve the diffusion coefficient D. When $D \to \infty$, we expect and find that the mean capture time $\tau \to 0$. Note that this is the same limit approached by τ_∞, the mean capture time for the model where traps are infinitely long-lived. This is not surprising since as $D \to \infty$, particles can travel long distances during the cycle time of a trap and, as indicated earlier, the infinitely long-lived trap model suffices in this case.

As the diffusion coefficient $D \to 0$, the mean capture time τ tends, as expected, to infinity. When we consider the recycling model where traps appear at random locations, we will consider the possibility that traps appear where particles are already present and capture those particles instantly. Then even particles with a diffusion coefficient $D = 0$ have a finite mean capture time.

The model breaks down if the trap locations are not diffuse, in particular if λ_2 is small relative to λ_1. Then the outer boundary about a trap location approaches the trap itself ($b \to a$). Most trap locations correspond to closed traps, and these stay closed a long time. But the outer boundary condition $w_1(b) = w_2(b)$ says that particles on the boundary of a closed trap are trapped just as rapidly as those on an open trap boundary. This results in Eqs. 15–17, which give a faster mean capture time than is physically reasonable. A sufficient condition for the applicability of the model is that the spacing between traps be large relative to the distance a particle diffuses during the cycle time of a trap. The trap spacing is measured approximately by $1/\sqrt{P_T}$, where the trap location density P_T is related to the density P of open traps by Eq. 9a. The mean square distance a diffusing particle travels in the mean cycle time of a trap is $4D(1/\lambda_1 + 1/\lambda_2)$. Then the model is valid when:

$$1 \gg \sqrt{4DP/\lambda_1}\,(\lambda_1 + \lambda_2)/\lambda_2. \quad (21)$$

This inequality holds when the effect of the opening and closing of the trap is important, i.e., when the particle "sees" the trap opening and closing many times before it is trapped.

Inequality 21 is a sufficient but not a necessary condition for the applicability of the model. We have seen that in some of the limits where inequality 21 fails to hold, i.e., $D \to \infty$ or $\lambda_1 \to 0$, the model still works, in the sense that it approaches the appropriate limiting model where traps have infinite lifetimes.

Note that in the limit as $P \to 0$ (i.e., in the case of an infinitely dilute system of traps), inequality 21 is satisfied for all finite parameter values. Unfortunately, in two dimensions one cannot go to this limit and still obtain solutions to the steady state Eqs. 10a and 10b. In three dimensions, the infinitely dilute case is the usual model considered, and there is no problem in taking the limit of k_+ as $b \to \infty$. In Appendix C, we give the expression for τ in three dimensions and show that it leads to the same expression for k_+ obtained by Szabo et al. (23).

Steady State Flux Method. We define $C_1(r)$ and $C_2(r)$ to be, respectively, the concentrations of particles at position r with the trap open and closed, and S to be the number of particles per second per unit area inserted into the annulus between a and b. Then, in the steady state,

$$D\nabla^2 C_1(r) - \lambda_1 C_1(r) + \lambda_2 C_2(r) = -\lambda_2 S/(\lambda_1 + \lambda_2); \quad (22a)$$

$$D\nabla^2 C_2(r) + \lambda_1 C_1(r) - \lambda_2 C_2(r) = -\lambda_1 S/(\lambda_1 + \lambda_2), \quad (22b)$$

since $\lambda_i/(\lambda_1 + \lambda_2)$ is the probability that a trap is open ($i = 2$) or closed ($i = 1$). Again we assume that $r = a$ is an absorbing boundary when the trap is open, and a reflecting boundary when the trap is closed. (The equations equivalent to Eqs. 22a and 22b for three dimensions have been obtained by Szabo et al. [23].) The outer boundary conditions analogous to those derived for the mean capture times are:

$$\frac{d}{dr}[C_1(r) + C_2(r)]\bigg|_{r=b} = 0; \quad (23a)$$

$$\lambda_1 C_1(b) = \lambda_2 C_2(b). \quad (23b)$$

To calculate k_+ from Eq. 8, we need to find:

$$\langle C \rangle = (2/b^2) \int_0^b rC(r)\mathrm{d}r, \tag{24}$$

where

$$C(r) = C_1(r) + C_2(r). \tag{25}$$

In the steady state, the total flux at the trap location equals the total number of particles inserted per second between a and b, i.e., flux $= \pi(b^2 - a^2)S$. To obtain the flux per open trap, the flux per trap location must be multiplied by $P_T/P = (\lambda_1 + \lambda_2)/\lambda_2$. Then k_+ is found, following Eq. 8, as the flux per open trap divided by the average particle concentration $\langle C \rangle$:

$$k_+ = \frac{\lambda_1 + \lambda_2}{\lambda_2} \frac{\pi(b^2 - a^2)S}{\langle C \rangle}. \tag{26}$$

When the equations for C_1 and C_2 are solved subject to the appropriate boundary conditions and Eq. 26 is evaluated at $b = b_r$, the resulting expression for k_+ is the same as that given by Eq. 20, using the mean capture time method.

The equivalence between the two methods for calculating k_+ can be demonstrated without solving any equations by noting that Eqs. 22a and 22b and the boundary conditions for these equations can be transformed into Eqs. 10a and 10b and the appropriate boundary conditions for those equations, by letting

$$C_1(r)/S = \lambda_2 w_1(r)/(\lambda_1 + \lambda_2);$$
$$C_2(r)/S = \lambda_1 w_2(r)/(\lambda_1 + \lambda_2).$$

With these identities, it follows from Eq. 14 that $\tau = \langle C \rangle b^2/S(b^2 - a^2)$, and therefore that Eqs. 26 and 20 for k_+ agree.

Fixed-Location Model with Diffusion into Closed Traps. In the preceding sections, the diffusion-limited forward rate constant for the interaction of diffusing receptors with traps alternating between closed and open states was calculated under the assumption that the particles were excluded from closed traps. The boundary of a closed trap was taken to be reflecting. We now consider the case where particles may diffuse over the surface of a closed trap; i.e., we assume that when the trap is closed, the membrane surface between $r = 0$ and $r = a$ is identical with the surface between a and b. We further assume that particles in a closed trap are captured when the trap opens. We still assume that particles are created uniformly and at a constant rate S in the annulus about a trap but not in closed or open traps. Then the equations and outer boundary conditions used to find the mean capture time $w(r)$ or the particle concentration $C(r)$ remain valid for $a \leqq r \leqq b$, but we must now give equations for w_1 and w_2 or C_1 and C_2 inside a trap, i.e., for $0 \leqq r \leqq a$, and impose appropriate continuity conditions at $r = a$. For $0 \leqq r < a$, the mean capture times $w_1(r)$ and $w_2(r)$ for particles in open or closed traps satisfy:

$$w_1(r) = 0; \tag{27a}$$

$$D\nabla^2 w_2(r) - \lambda_2 w_2(r) = -1. \tag{27b}$$

Eqs. 10a, 10b, 13a, 13b, 27a, and 27b for w_1 and w_2 have a unique solution for $0 \leqq r \leqq b$ under the additional assumptions that w_1, w_2, and $\mathrm{d}w_2/\mathrm{d}r$ are continuous at $r = a$ and that w_2 is finite at $r = 0$. The mean capture time τ for particles starting at random locations between $r = a$ and $r = b$ is:

$$\tau = \tau_\infty + \tau_2, \tag{28}$$

where

$$\tau_2 = \frac{\lambda_1}{\lambda_1 + \lambda_2} w_2(a); \tag{29a}$$

$$w_2(a) = \left[\frac{(b^2 - a^2)}{2Da\alpha_2} \frac{I_0(\alpha_2 a)}{I_1(\alpha_2 a)} + \frac{1}{\lambda_2}\right] \Bigg/ \left[1 + \left(\frac{\lambda_2}{\lambda_1 + \lambda_2}\right)^{1/2} \frac{I_0(\alpha_2 a)}{I_1(\alpha_2 a)} \frac{\sigma_{01}}{\delta_{00}}\right]; \tag{29b}$$

$$\alpha_2 = (\lambda_2/D)^{1/2}; \tag{30}$$

and τ_∞, σ_{01}, and δ_{00} are given by Eqs. 16, 19a, 19b. Then the diffusion-limited forward rate constant k_+ is given by $k_+ = 1/\tau P$, evaluated at $b = b_r$.

The same expression for k_+ can be derived using the flux method. For $0 \leqq r < a$, the concentrations $C_1(r)$ and $C_2(r)$ of particles in open or closed traps:

$$C_1(r) = 0;$$
$$D\nabla^2 C_2(r) - \lambda_2 C_2(r) = 0.$$

The right side of the above equation equals zero because we assume particles are only inserted into the membrane at a constant rate S between a and b. The only source of particles between 0 and a is from diffusion across the boundary $r = a$ when the trap is closed.

Random Reappearance Model

We now know the diffusion-limited forward rate constant for the interaction of a diffusing particle with an infinitely long-lived trap (model 1, reference 13), and with a trap that disappears and then reappears at the original location (model 2). We turn next to model 3, in which traps disappear from one location and then reappear at a random location.

We assume that when traps appear at random locations, the initial particle distribution is uniform. We can envision at least two possible ways to model the uniform particle distribution a trap encounters when it appears. One possibility is that when a trap first arises, particles are distributed uniformly in an annulus about the trap, but not inside

it. Here we picture the return of the trap as the insertion of both a clathrin coat and, above it, new membrane from 0 to a that is devoid of receptors. Alternatively, we can assume that initially particles are distributed uniformly in a region including the trap itself, and the particles inside the trap are captured instantly. Here we picture the clathrin coat rapidly forming between 0 and a below a randomly selected piece of plasma membrane that already contains diffusing receptors. These receptors are instantly trapped by the formation of the clathrin coat. Below, we will calculate, for both cases, the diffusion-limited forward rate constant.

In both cases, we assume that traps remain at a location for an exponentially distributed length of time with mean $1/\lambda_1$ (i.e., they disappear at rate λ_1). Also in both cases, particles are created only outside of traps, at a rate S.

Traps Empty Initially. As before, particles are assumed to diffuse with two-dimensional diffusion coefficient D in an annulus of outer radius b about a trap of radius a. In this case, the outer radius b is related to the trap density P by Eq. 6; i.e., $P = 1/\pi b^2$.

Again, the diffusion-limited forward rate constant can be calculated either by the flux method or by the mean capture time method. The flux calculation begins with the following equation for the concentration of particles a distance r ($a \leqq r \leqq b$) from the trap center, at time t ($t \geqq 0$) after the pit appears:

$$\frac{\partial C}{\partial t} = D\nabla^2 C - \lambda_1 C + S. \tag{31}$$

The second term of Eq. 31 reflects the effect of the disappearance of traps. When a trap disappears, particles that were a distance r from its center are no longer a distance r from the center of a trap. In effect, these particles are redistributed, since they are now located randomly with respect to the other traps. The other terms in Eq. 31 reflect changes in particle concentration caused by particle diffusion and creation. The creation rate S includes the replenishment of particles that are redistributed after traps disappear.

In Wofsy and Goldstein (15), we find a steady state solution $C(r)$ to Eq. 31 subject to the boundary conditions

$$C(a) = 0;$$
$$\left.\frac{\partial C}{\partial r}\right|_{r=b} = 0,$$

and use it in connection with Eq. 8 to calculate k_+. We will focus here on the mean capture time calculation, because the methods differ from those used in the cases already presented and are most easily extended to the next case, where traps reappear at random locations and capture particles already present at the trap site.

To calculate k_+ by the mean capture time method, we find:

$$\tau = \frac{1}{(b^2 - a^2)} \int_a^b 2r \int_0^\infty t h(r, t) \mathrm{d}t \mathrm{d}r, \tag{32}$$

where for each r ($a \leqq r \leqq b$), $h(r, t)$ is the probability density for the capture time t of a particle starting a distance r from the center of a trap. Eq. 32 averages the mean capture times given fixed starting positions r, over a uniform distribution of starting positions in the annulus between $r = a$ and $r = b$.

The density $h(r, t)$ is related to the probability density $p(r, t)$ for the time t that a particle takes to reach the trap when it starts a distance r from an infinitely long-lived trap. In the case of the transient trap, $p(r, t)\mathrm{d}t$ can be thought of as the probability that a particle initially at r reaches the trap location between times t and $t + \mathrm{d}t$. The probability that the trap is still there at time t is $e^{-\lambda_1 t}$. It follows that:

$$h(r, t)\mathrm{d}t = p(r, t)e^{-\lambda_1 t}\,\mathrm{d}t + \int_0^t \lambda_1 e^{-\lambda_1 x} \left[1 - \int_0^x p(r, u)\mathrm{d}u\right] \int_a^b [2r' h(r', t - x)\mathrm{d}r'/(b^2 - a^2)]\mathrm{d}x\,\mathrm{d}t. \tag{33a}$$

That is, a particle will be captured at time t if it reaches the trap's location and finds the trap still there; or if the trap disappears at some time $x < t$, before the particle reaches it, and the particle, starting fresh at a random position r' with respect to some other trap, takes the remaining time $t - x$ to be captured.[1] Averaging Eq. 33a over r, $a \leqq r \leqq b$ yields:

$$h(t) = p(t)e^{-\lambda_1 t} + \int_0^t \lambda_1 e^{-\lambda_1 x} \left[1 - \int_0^x p(u)\mathrm{d}u\right] h(t - x)\mathrm{d}x, \tag{33b}$$

where $h(t)$ and $p(t)$ denote the spatial means of $h(r, t)$ and $p(r, t)$ when particles are distributed uniformly over the annulus between $r = a$ and $r = b$. Then, taking Laplace transforms in Eq. 33b and evaluating the mean capture time τ as the negative of the derivative at 0 of the Laplace transform of h, we find:

$$\tau = (1 - f)/(\lambda_1 f), \tag{34}$$

where f is the probability that a particle starting a random distance from a trap will hit the trap before it disappears; i.e.,

$$f = \int_a^b 2r \int_0^\infty p(r, t)e^{-\lambda_1 t}\mathrm{d}t\,\mathrm{d}r/(b^2 - a^2). \tag{35}$$

[1] In reference 14, we attempted to calculate τ and k_+ for the random reappearance model. However, that calculation is in error because we calculated the conditional mean capture time for those receptors captured by a single coated pit before it disappears; i.e., we did not include the second term in Eq. 33a.

In reference 14 we showed that:

$$\int_0^\infty p(r,t)e^{-\lambda_1 t}dt = [I_1(\alpha b)K_0(\alpha r) + K_1(\alpha b)I_0(\alpha r)]/\sigma_{10}, \quad (36)$$

where σ_{10} is given by Eq. 19a and $\alpha = \sqrt{\lambda_1/D}$. Therefore, from Eqs. 35 and 36, we find that

$$f = 2a\delta_{11}/[(b^2 - a^2)\alpha\sigma_{10}]. \quad (37)$$

Substituting into Eq. 7 the trap density $P = 1/\pi b^2$ and the mean absorption time τ given by Eq. 34, we obtain

$$k_+ = \pi b^2 \lambda_1 f/(1 - f). \quad (38)$$

Traps Capture Particles Initially. Now we consider the possibility that the trap forms in a region where diffusing particles are already present and captures these particles instantly (e.g., if coated pits are formed by the rapid assembly of a clathrin coat below a portion of the plasma membrane containing diffusing receptors). We still assume that particles are created only outside traps. Then Eq. 33b for the mean capture time density $h(t)$ of a particle starting at a random location in the annulus about a trap is replaced by:

$$h(t) = p(t)e^{-\lambda_1 t} + \frac{a^2}{b^2}\lambda_1 e^{-\lambda_1 t}\left[1 - \int_0^t p(u)du\right] + \left(1 - \frac{a^2}{b^2}\right)\int_0^t \lambda_1 e^{-\lambda_1 x}\left[1 - \int_0^x p(u)du\right]h(t - x)dx. \quad (39)$$

The first term of the right side of Eq. 39 is associated with the probability that a particle will reach the trap at time t, before it has disappeared. The second term reflects the probability that the particle will not reach the trap it sees initially, but will be absorbed instantly at time t when the original trap disappears and the next trap appears over the particle's location. The final term reflects the probability of the alternative; i.e., the particle does not reach the first trap before it disappears and is not covered by the next trap when it appears, but reaches some trap by some route at time t.

The Laplace transform procedure used to find the mean capture time τ in the preceding section now yields:

$$\tau = \frac{1 - f}{\lambda_1\left[f + \frac{a^2}{b^2}(1 - f)\right]}, \quad (40)$$

where f, given by Eq. 37, is the probability that a particle reaches the original trap before it disappears. Then

$$k_+ = \pi a^2 \lambda_1 + \pi b^2 \lambda_1 f/(1 - f). \quad (41)$$

Note that as $D \to 0$, $f \to 0$, and $k_+ \to \pi a^2 \lambda_1$. Since in this limit particles are stationary, the only particles that are trapped are those between 0 and a when the trap appears. The flux into a single trap is therefore $\pi a^2 \langle C \rangle \lambda_1$; i.e., every $1/\lambda_1$ min, $\pi a^2 \langle C \rangle$ particles are trapped, and therefore $k_+ = \pi a^2 \lambda_1$ is what we expect in this limit. From Eq. 41 we see that k_+ is the sum of two terms, the flux caused by particles captured when the trap appears, $\pi a^2 \lambda_1$, and the flux caused by particles diffusing into the trap, given by Eq. 38.

In the same limit, the fraction ϕ of particles in traps can be found directly in terms of average particle concentrations or indirectly in terms of k_+, providing an additional check on the consistency of the model. When $D = 0$, the number of particles in a trap at any time is the number captured initially, $\pi a^2 \langle C \rangle$. The total number in the trap plus surrounding annulus, when the trap has been present for a time t, is the initial number $\pi b^2 \langle C \rangle$ plus the number synthesized in time t: $\pi(b^2 - a^2)St$, where $S = a^2 \langle C \rangle \lambda_1/(b^2 - a^2)$. Averaging the total number of particles in and about a trap with respect to the trap lifetime distribution, we obtain:

$$\int_0^\infty (\pi b^2 \langle C \rangle + \pi a^2 \langle C \rangle \lambda_1 t)(\lambda_1 e^{-\lambda_1 t})dt = \pi(b^2 + a^2)\langle C \rangle.$$

Then, when $D = 0$, the fraction of particles in traps is $\phi = \pi a^2 \langle C \rangle / \pi(b^2 + a^2)\langle C \rangle$, or

$$\phi = \frac{a^2}{b^2 + a^2}. \quad (42)$$

The other way to obtain Eq. 42 is to calculate ϕ as the ratio of the average time a receptor spends in a trap to the average time a receptor spends on the cell surface; i.e.,

$$\phi = \frac{1/\lambda_1}{(1/\lambda_1) + (1/k_+ P)}. \quad (43)$$

Substituting into Eq. 43 $k_+ = \pi a^2 \lambda_1$ (when $D = 0$) and $P = 1/\pi b^2$, we obtain Eq. 42.

RESULTS

In the preceding section, for various models of coated pit dynamics, we have obtained expressions for the forward rate constant and the mean capture time, i.e., the average time it takes a recycling receptor molecule, once it appears on the cell surface, to encounter a coated pit. We now evaluate these expressions for typical parameter values and discuss the implications of the numerical values we obtain. We will concentrate on the endocytosis of LDL receptors on human fibroblasts, since all the parameter values we need for our calculations have been determined for this system. These values are summarized in Table I.

We compare in Fig. 1 how the predicted mean capture time changes with the receptor diffusion coefficient for (*a*) infinitely long-lived traps (Eq. 16 with b given by Eq. 6), (*b*) traps that disappear and reappear at the same location (Eq. 28), and traps that disappear and reappear at random locations (Eq. 40). For models 2 and 3, we assume that when a trap forms in a region where there are diffusing receptors, it captures these receptors instantly. For rates of trap opening and closing estimated from experiments with

TABLE I
PARAMETER VALUES FOR LDL RECEPTORS AND COATED PITS ON HUMAN FIBROBLASTS

Parameter	Symbol	Value
Percentage of LDL receptors in coated pits at 4°C	$\phi_{4°C}$	69 ± 7%
Ratio of LDL receptors in coated pits to LDL receptors out of coated pits at 4°C	$\rho_{4°C}$	2.2 ± 0.8
Surface density of coated pits at 4°C	$P_{4°C}$	0.58 ± 0.05/μm^2
Ratio of the number of coated pits on the cell surface at 37°C to the number at 4°C	r	0.53 ± 0.10
Surface density of coated pits at 37°C	P	0.31 ± 0.09/μm^2
Characteristic radius associated with the coated pit density	b	1.0 ± 0.2 μm
Radius of a coated pit	a	0.10 ± 0.05 μm
Fraction of surface area covered by coated pits at 37°C	A	0.01
Diffusion coefficient of LDL receptors between 27–28°C	D	(4.5 ± 1.5) × 10^{-11} cm^2/s
Rate constant for the internalization (closing) of coated pits at 37°C	λ_1	≥0.19 ± 0.05 min^{-1}
Rate constant for the recycling (opening) of coated pits at 37°C	λ_2	≤0.17 ± 0.10 min^{-1}

We obtained $\phi_{4°C}$ by averaging (± SEM) the eight published measurements given in the literature, which ranged from 49 to 95% (4, 27–29). We obtained $\rho_{4°C} = R_p/R$ from the formula $\rho_{4°C} = \phi_{4°C}/(1 - \phi_{4°C})$. Note that small changes in $\phi_{4°C}$ can lead to large changes in $\rho_{4°C}$. We assumed $\phi = \phi_{4°C}$ and $\rho = \rho_{4°C}$, where ϕ and ρ are the 37°C values of these parameters. (For a discussion of this point see reference 14.) $P_{4°C}$ was determined by Orci et al. (28) to be 0.52 ± 0.05/μm^2 for human fibroblasts from a normal individual and 0.63 ± 0.06/μm^2 for human fibroblasts from a patient with the receptor-negative form of homozygous FH. 50 cells were sampled in each determination. We took $P_{4°C}$ to be the average of these measurements. r is the average of the determinations of two different experimental groups (24, 25). $P = rP_{4°C}$ and $b = 1/\sqrt{\pi P}$. On human fibroblasts, a has been reported to range from 0.05 to 0.25 μm (4, 28, 30, 31). The most complete study was performed on mouse fibroblasts by Heuser (31), in which a ranged from 0.05 to 0.15 μm with an average value of 0.11 μm and a median value of 0.095 μm. A was calculated by taking $A_{4°C} = \pi a^2 P$. Barak and Webb (16), from fluorescence photobleaching measurements, determined that D, the average diffusion coefficient of a bound LDL receptor on the surface of a JD mutant human fibroblast between 27 and 28°C was = (4.5 ± 1.5) × 10^{-11} cm^2/s. The LDL receptors on JD cells lack the ability to incorporate into coated pits (32). A detailed discussion of how we estimated λ_1 and λ_2 appears in reference 15. Briefly, λ_1 was estimated in two ways, from experiments where the number of coated pits was measured as a function of time after cells were warmed from 4 to 37°C (24, 25), and from experiments at 37°C where both the total amount of ^{125}I-LDL internalized in 5 h and the total amount of ^{125}I-LDL bound to the cell surface were determined (32, 33). We estimated λ_2 by assuming that the reason there are more coated pits on the surface at 4 than at 37°C is that the stable state of all coated structures at 4°C is the open structure, the coated pit. Then $\lambda_2 = \lambda_1(1 - r)/r$ (33).

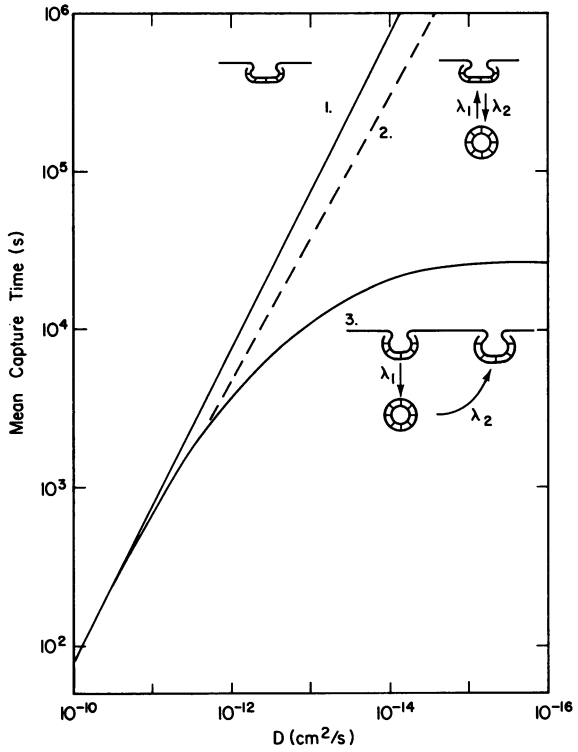

FIGURE 1 The mean capture time τ as a function of the receptor diffusion coefficient D as predicted by model 1: infinitely long-lived traps; model 2: traps that disappear and reappear at the same location; and model 3: traps that disappear and reappear at random locations. For models 2 and 3, we assume that when a trap of radius a forms, it instantly captures all receptors in the region between $r = 0$ and $r = a$. The values of parameters used in calculating these curves are characteristic of human fibroblasts: $\lambda_1 = \lambda_2 = 0.2$ min^{-1}, $a = 0.10$ μm, and $b = 1.0$ μm. This value of b corresponds to $P = 0.31/\mu m^2$.

human fibroblasts, $\lambda_1 \cong \lambda_2 = 0.2$ min^{-1}, we see from Fig. 1 that for $D \geq 1 \times 10^{-11}$ cm^2/s, the predictions of the three models differ by ≤12%. This is because receptors are trapped in times that are shorter than the lifetime of a coated pit ($1/\lambda_1 = 5$ min), and therefore the dynamics of the coated pits have little influence on the rate at which receptors are trapped.

For most mobile cell surface receptors, $D \cong (5 \times 10^{-10} - 5 \times 10^{-11})$ cm^2/s. We predict for this range of D values that the mean capture time $\tau \equiv (1.6 \times 10^1 - 1.6 \times 10^2)$ s, providing that the capture process is diffusion limited. For the LDL receptor on JD cells, a mutant human fibroblast cell line, $D = (4.5 \pm 1.5) \times 10^{-11}$ cm^2/s (16). Model 1, infinitely long-lived traps, predicts that $\tau = 1.78 \times 10^2$ s and $k_+ = 1.82 \times 10^{-10}$ cm^2/s, while model 3, traps that disappear and reappear at random locations, predicts that $\tau = 1.73 \times 10^2$ s and $k_+ = 1.88 \times 10^{-10}$ cm^2/s. These theoretical values for k_+ are consistent with the experimental estimate given in Eq. 5 of $k_+ \geq 2.3 \times 10^{-10}$ cm^2/s.

Although the details of coated pits recycling on human fibroblasts have little effect on the trapping rates of mobile

receptors ($D \geqq 1 \times 10^{-11}$ cm^2/s), when diffusion coefficients of receptors are measured by fluorescence photobleaching recovery techniques, almost always a fraction of the receptors are found to be "immobile." Here "immobile" means that their diffusion coefficients are smaller than some critical value, below which their motions cannot be detected in the time of the experiment. For example, Barak and Webb (16) found that at 27–28°C, 40 ± 20% of the LDL receptors on JD cells were immobile in the sense that $D \leqq 2.0 \times 10^{-11}$ cm^2/s for these receptors. As D approaches zero, τ becomes infinite for models 1 and 2, but approaches a finite value for model 3. In model 3, coated pits return to random locations; if a receptor cannot diffuse to a coated pit, a coated pit can still go to (grow up around) the receptor. For human fibroblasts, i.e., $\lambda_1 = 0.2$ min^{-1}, model 3 predicts that $\tau = 7.9$ h for a receptor with $D = 0$ that is recognized by a coated pit. (See the discussion following Eq. 41.)

Willingham and Pastan (20) have suggested that coated pits close and open much faster than the rates given in Table I. They estimated that coated pits stay closed for ~7 s, and that in the steady state, two-thirds of the coated structures are open and one-third are closed. Thus, if the closed state has a lifetime of 7 s, the coated pit has a lifetime of 14 s. This corresponds to $\lambda_1 = 4.29$ min^{-1} and $\lambda_2 = 8.57$ min^{-1} In Fig. 2 we present the model predictions for these parameters. Model 3 now predicts that $\tau = 22$ min for a receptor with $D = 0$. Because the lifetime of the coated pit is so short, for $D < 10^{-10}$ cm^2/s, the dynamics of coated pit recycling strongly influence the predicted mean capture times. Model 2 looks very much like model 1, infinitely long-lived coated pits, because the coated pits stay closed for such a short period of time (7 s) that there is little change in the distribution of receptors around the coated pit during the time it is closed.

One of the most striking observations about LDL receptors is that on human fibroblasts ~70% of the receptors are found in coated pits in the absence of LDL (4, 27–29). In Fig. 3 we show the theoretical prediction for the fraction of receptors in coated pits as a function of the rate at which coated pits are internalized, λ_1, for three different receptor diffusion coefficients. We assume that when a receptor hits a coated pit, it is trapped and remains trapped for the lifetime of the coated pit; the longer a coated pit stays open (the smaller λ_1), the larger the fraction of receptors that will be found in coated pits. The curve with $D = 4.5 \times 10^{-11}$ cm^2/s corresponds to the LDL receptor. For $\lambda_1 = 0.19$ min^{-1}, the estimated value for human fibroblasts (see Table I), the predicted fraction in coated pits is 65%, which is very close to the experimentally determined value of 69 ± 7%. If on human fibroblasts λ_1 were much larger than 0.19 min^{-1}, so that the lifetime of a coated pit were much shorter than 5 min, then the predicted fraction of LDL receptors in coated pits would be much smaller than 65%; diffusion would be unable to account for the observed high fraction of LDL receptors in coated pits.

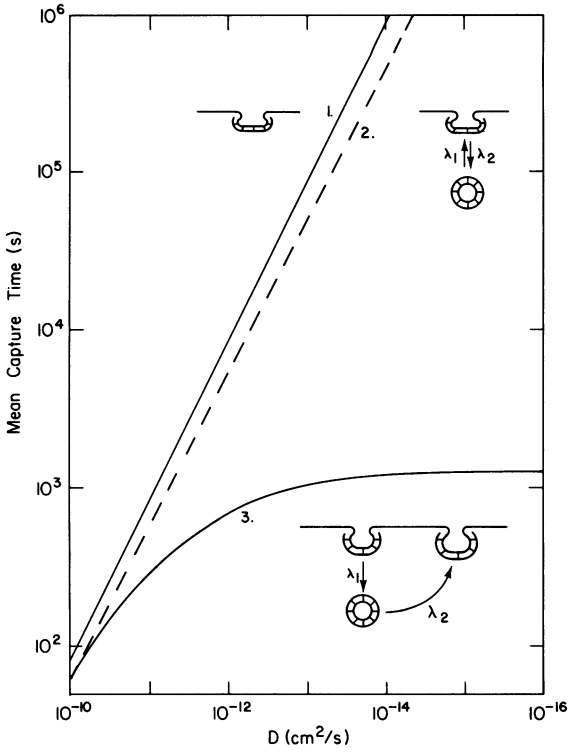

FIGURE 2 The mean capture time τ as a function of the receptor diffusion coefficient D as predicted by model 1: infinitely long-lived traps; model 2: traps that disappear and reappear at the same location; and model 3: traps that disappear and reappear at random locations. For models 2 and 3, we assume that when a trap of radius a forms, it instantly captures all receptors in the region between $r = 0$ and $r = a$. The rates of opening and closing of the traps were taken to be much faster than for human fibroblasts. For the calculation, we took $\lambda_1 = 4.29$ min^{-1}, $\lambda_2 = 8.57$ min^{-1}, $a = 0.10$ μm, and $b = 1.0$ μm. This value of b corresponds to $P = 0.31/\mu$m^2.

A more typical value for the diffusion coefficient of a receptor is $\sim 5 \times 10^{-10}$ cm^2/s. For example, $D = (3 - 5) \times 10^{-10}$ cm^2/s for the EGF receptor at 28°C on 3T3 mouse fibroblasts (34). From Fig. 3 we see that if receptors with this value of D are irreversibly trapped when they encounter a coated pit, 95% of them will be in coated pits on human fibroblasts ($\lambda_1 = 0.19$ min^{-1}). Since there is no evidence that EGF receptors aggregate in coated pits to this extent in the absence of EGF, they must not be irreversibly bound when they encounter a coated pit.

The bottom curve in Fig. 3 illustrates what we would expect for receptors in the immobile fraction with $D = 1.0 \times 10^{-12}$ cm^2/s. For $\lambda_1 = 0.19$ min^{-1}, only 7% of the receptors would be in coated pits.

In Fig. 4 we illustrate how increasing λ_1, or equivalently decreasing the lifetime of a coated pit, increases the forward rate constant when coated pits recycle to random locations (model 3). The slower the diffusion coefficient of the receptor is, the greater the effect of decreasing λ_1 is on k_+. When $\lambda_1 = 0.2$ min^{-1}, k_+ is twice as great as it would be if the coated pit were infinitely long-lived when $D = 1 \times$

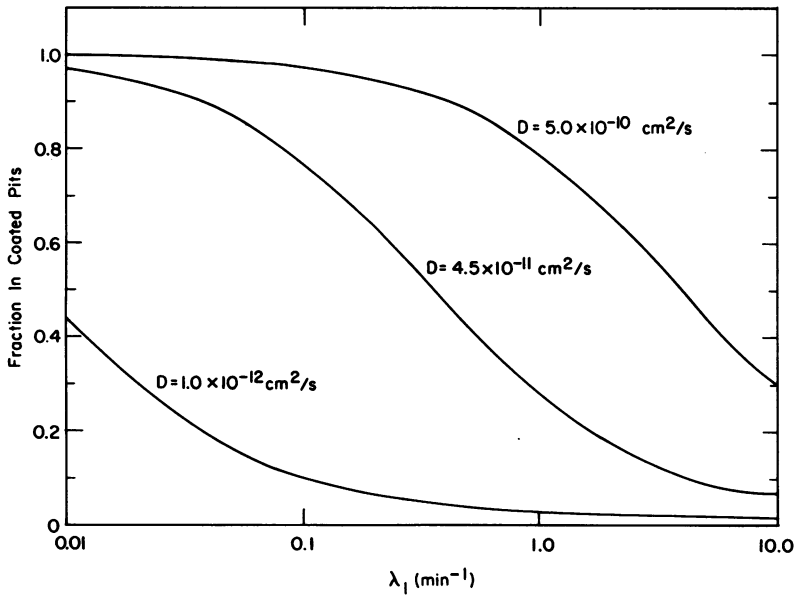

FIGURE 3 The predicted fraction of receptors in coated pits, ϕ, as a function of the rate of internalization of coated pits, λ_1, for three values of the receptor diffusion coefficient D. We assume that when a receptor reaches the outer radius of a coated pit, it is trapped and remains trapped for the lifetime of the coated pit. ϕ was calculated from Eqs. 41 and 43 for model 3, traps that disappear and reappear at random locations. We took $a = 0.10\ \mu m$ and $b = 1.0\ \mu m$. For $D = 5.0 \times 10^{-10}\ cm^2/s$ and $D = 4.5 \times 10^{-11}\ cm^2/s$, models 2 and 3 make essentially the same predictions for $\lambda_2 = 0.2\ min^{-1}$ (not shown).

10^{-12} cm^2/s, but only 2% greater when $D = 4.5 \times 10^{-11}$ cm^2/s. The predicted values of k_+ for model 2 (not shown) are always less than the predicted values for model 3 and greater than model 1. As $\lambda_2 \rightarrow \infty$, any coated pit that closes instantly returns to the surface; model 2 becomes equal to model 1. As $\lambda_1 \rightarrow \infty$, the probability of a coated pit closing and then reopening at the same location becomes negligibly small; model 2 should become equal to model 3. However, in this limit, the inequality given in 21 is not satisfied and our analytic results for model 2 are no longer valid.

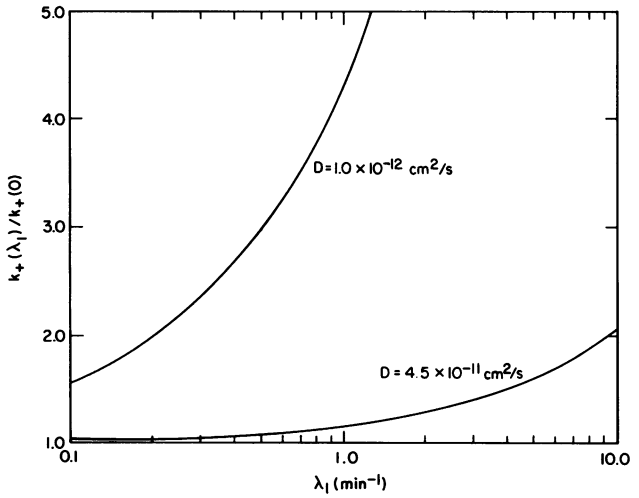

FIGURE 4 The effect of the rate of coated pit internalization, λ_1, on the diffusion-limited forward rate constant $k_+(\lambda_1)$ for the interaction of a receptor with diffusion coefficient D, with a coated pit. $k_+(0)$ is the value of k_+ when the coated pits are infinitely long-lived, $\lambda_1 = 0$. The calculations are for model 3, Eq. 40, traps that disappear and reappear at random locations. We took $a = 0.10\ \mu m$ and $b = 1.0\ \mu m$. For $D = 4.5 \times 10^{-11}$ cm^2/s and $\lambda_2 = 0.2\ min^{-1}$, there is very little difference in the predictions made by models 2 and 3.

CONCLUSION

Coated pits trap receptors and then close off to transport them across the plasma membrane. Most of the details of how the coated structure recycles to maintain a steady state concentration of coated pits are unknown. As viewed from the surface, recycling coated pits can only do two things: disappear and then at a later time reappear at approximately the same location, or disappear and then at a later time reappear at a different location. We have investigated theoretically the consequences of those two possibilities by deriving for both recycling strategies analytic expressions for the mean capture times, τ, and forward rate constants, k_+, for the trapping of cell surface receptors by coated pits. We have found that for parameter values characterizing human fibroblasts (Table I), the way in which the coated pit returns to the surface has a negligible effect on the values of τ and k_+ for mobile receptors ($D \geqq 1 \times 10^{-11}$ cm^2/s). This is because the lifetime of a coated pit is ~5 min on these cells, while the average time a receptor spends on the cell surface before being captured is somewhat less. Thus, an "average receptor" never gets to experience the effects of the coated pit recycling. We predict, for example, that on human fibro-

blasts, for $D = 5 \times 10^{-10}$ cm^2/s, $\tau = 16$ s, while for $D = 4.5 \times 10^{-11}$ cm^2/s, the measured value of the LDL receptor, $\tau = 2.9$ min.

For immobile receptors ($D < 1 \times 10^{-11}$ cm^2/s), however, the two types of coated pit recycling strategies make substantially different predictions. We predict that on human fibroblasts, for a receptor with $D = 1.0 \times 10^{-12}$ cm^2/s, $\tau = 68$ min if coated pits recycle to random locations, and $\tau = 79$ min if they return to the same locations. If the lifetime of the coated pit were smaller, as may be the case on some cell types (20), the differences would be more pronounced. For example, if the lifetime of a coated pit were 1 min rather than 5 min, for $D = 1.0 \times 10^{-12}$ cm^2/s, $\tau = 31$ min for coated pits that recycle to random locations and $\tau = 63$ min for coated pits that return to the same location.

On human fibroblasts, ~70% of the LDL receptors are in coated pits (4, 27–29). We predict for the parameter values of Table I that if LDL receptors are trapped when they encounter a coated pit and remain trapped for the lifetime of the pit, this fraction should equal 65%. Considering the range of errors in the parameter values of Table I, theory and experiment are in good agreement. This means that the experimental observations are consistent with a model in which LDL receptors are randomly inserted into the cell membrane, move by pure diffusion to coated pits, and are irreversibly trapped by the coated pits. Since LDL has a much lower diffusion coefficient than most other mobile receptors, pure diffusion as the mechanism for a receptor getting to a coated pit is consistent with the observed rapid rates at which receptor mediated endocytosis takes place in general.

APPENDIX A

Here we outline a heuristic derivation of Eqs. 10a and 10b based on a random walk approximation. The equations can be derived rigorously using the theory of operators characterizing Markov processes. (Szabo et al. [35] discusses the general forms of the operators characterizing diffusion processes. The joint process consisting of the position of the diffusing particle and the state of the trap is a random evolution. The operator theory for random evolutions was developed by Griego and Hersh [36].)

We consider a symmetric random walk on a square lattice with a step size δ and a time unit Δt. As δ and Δt approach 0 in the relation $\delta^2 = 4D\Delta t$, the corresponding random walks converge to the two-dimensional diffusion process with diffusion coefficient D under investigation. Then $w_1(x, y)$, the mean capture time for a diffusing particle starting at position (x, y) with the trap open, is approximately equal to the duration Δt of the first step in the random walk plus the remaining time until the particle is captured, averaged over the particle's position and the state of the trap after the first step. Since the probability that the trap closes in a small time Δt is approximately $\lambda_1 \Delta t$ and the particle is equally likely to move to any of its four nearest neighbors, we find:

$$w_1(x, y) \simeq \Delta t + (1 - \lambda_1 \Delta t)\frac{1}{4}[w_1(x + \delta, y) + w_1(x - \delta, y) + w_1(x, y + \delta) + w_1(x, y - \delta)]$$

$$+ \lambda_1 \Delta t \frac{1}{4}[w_2(x + \delta, y) + w_2(x - \delta, y) + w_2(x, y + \delta) + w_2(x, y - \delta)],$$

where $w_2(x, y)$ is the mean capture time for a particle at position (x, y) with the trap closed. Expanding w_1 and w_2 in Taylor series about (x, y), we obtain

$$0 \simeq \Delta t + \frac{1}{4}\delta^2\left[\frac{\partial^2 w_1(x, y)}{\partial x^2} + \frac{\partial^2 w_2(x, y)}{\partial y^2}\right] - \lambda_1 \Delta t w_1(x, y) + \lambda_1 \Delta t w_2(x, y). \quad \text{(A1)}$$

Using the relation $\delta^2 = 4D\Delta t$, dividing by Δt and taking the limit in Eq. A1 as Δt approaches 0 yields Eq. 10a:

$$D\nabla^2 w_1 - \lambda_1 w_1 + \lambda_1 w_2 + 1 = 0.$$

Similar arguments lead to Eq. 10*b*.

APPENDIX B

In this section, we outline the solution of Eqs. 10a and 10b for mean capture times w_1 and w_2 for particles starting about open and closed traps subject to the boundary conditions Eqs. 11a, 11b, 13a, and 13b.

Multiplying Eq. 10a by $\lambda_2/(\lambda_1 + \lambda_2)$ and Eq. 10b by $\lambda_1/(\lambda_2 + \lambda_2)$ and adding the resulting equations leads to the following equation for the mean capture time $w(r)$ for particles starting a distance r from a trap in a random state:

$$D\nabla^2 w(r) = -1. \quad \text{(B1)}$$

Subject to the boundary condition Eq. 13a, i.e., $dw/dr = 0$ at $r = b$, Eq. B1 has the solution:

$$w(r) = w(a) + \frac{1}{4D}\left(2b^2 \ln\frac{r}{a} - r^2 + a^2\right). \quad \text{(B2)}$$

To find $w(a)$, we solve for w_1, using the fact that $\lambda_1 w_2 = (\lambda_1 + \lambda_2)w - \lambda_2 w_1$. Then Eq. 10a can be written as:

$$D\nabla^2 w_1 - (\lambda_1 + \lambda_2)w_1 = -(\lambda_1 + \lambda_2)w(r) - 1, \quad \text{(B3)}$$

where $w(r)$ is given by Eq. B2. The homogeneous equation

$$\nabla^2 y - (\lambda_1 + \lambda_2)y/D = 0$$

has the general solution:

$$y(r) = AI_0(r\alpha) + BK_0(r\alpha),$$

where $\alpha = [(\lambda_1 + \lambda_2)/D]^{1/2}$ and A and B are arbitrary constants. The general solution to Eq. B3 subject to the additional boundary condition 11*a*, i.e., $w_1(a) = 0$, is

$$w_1(r) = \frac{1}{4D}\left[2b^2 \ln\frac{r}{a} - r^2 + a^2\right] + A[I_0(r\alpha) - I_0(a\alpha)] + B[K_0(r\alpha) - K_0(\alpha a)]. \quad \text{(B4)}$$

An expression for w_2 can be found from the relation between w_1, w_2, and w.

Imposing the remaining boundary conditions, given by Eqs. 11b and 13b, to determine A and B, and using the resulting expressions for w_1 and

w_2 to find $w(r)$, we obtain

$$w(r) = \frac{1}{4D}\left(2b^2 \ln\frac{r}{a} - r^2 + a^2\right) + \frac{\lambda_1}{\lambda_2}\frac{(b^2 - a^2)}{2D\alpha a}\frac{\delta_{00}}{\sigma_{01}}, \quad \text{(B5)}$$

where δ_{00} and σ_{01} are as defined by Eqs. 19a and 19b.

APPENDIX C

In three dimensions, the solution to Eqs. 10a and 10b, subject to the boundary conditions Eqs. 11a and 11b, yields the following expressions for $w(r)$:

$$w(r) = \tau_a + \frac{b^3}{3D}\left(\frac{1}{a} - \frac{1}{b}\right) - \frac{1}{6D}(r^2 - a^2), \quad \text{(C1)}$$

where

$$\tau_a = \frac{\lambda_1}{\lambda_2}\frac{(b^3 - a^3)}{3Da}\left[(\alpha b - 1)e^{\alpha(b-a)} + (\alpha b + 1)e^{-\alpha(b-a)}\right] \Big/ \left[(\alpha a + 1)(\alpha b - 1)e^{\alpha(b-a)} - (\alpha a - 1)(\alpha b + 1)e^{-\alpha(b-a)}\right] \quad \text{(C2)}$$

and

$$\alpha = [(\lambda_1 + \lambda_2)/D]^{1/2}.$$

Averaging $w(r)$ over r, we find that $\tau = \tau_a + \tau_\infty$, where

$$\tau_\infty = \frac{b^3}{3Da} + \frac{a^2}{6D} - \frac{b^3(b^2 - a^2)}{2D(b^3 - a^3)} - \frac{(b^5 - a^5)}{10D(b^3 - a^3)}. \quad \text{(C3)}$$

In three dimensions, $P = 3/4\pi b^3$ and, therefore, from Eq. 7,

$$k_+^{-1} = (3/4\pi b^3)(\tau_\infty + \tau_a). \quad \text{(C4)}$$

In the infinite dilution limit, we have that

$$\lim_{b\to\infty} k_+^{-1} = k_D^{-1}\left[1 + \frac{\lambda_1}{\lambda_2}(1 + \alpha a)\right], \quad \text{(C5)}$$

where $k_D = 4\pi Da$. Eq. C5 agrees with the results of Szabo et al. (23). (In their notation, $\lambda_1 = a$, $\lambda_2 = b$, and $a = R$.)

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