Reports

Constraint of the Translational Diffusion of a Membrane Glycoprotein by Its External Domains

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The translational diffusion of wild-type and underglycosylated molecules of a membrane-integral glycoprotein the L^d class I major histocompatibility complex (MHC) antigen has been measured. The L^d mutant molecules, which lack one or more glycosylation sites, had larger translational diffusion coefficients, *D*, than did wild-type L^d molecules glycosylated at three sites. The increase in *D* is linear with loss of glycosylation. The highest value of *D* approaches that for translational diffusion of molecules constrained only by viscosity of the membrane lipid bilayer. These results indicate that the external portions of cell surface glycoproteins interact significantly with other nearby molecules.

DIFFUSION OF PROTEINS IN THE plane of the cell membrane, translational diffusion, is important in membrane organization (*1, 2*) and in membrane function (*3*). The translational diffusion coefficients of many membrane proteins have been measured with high precision by the technique of fluoresence photobleaching and recovery (FPR) (*4*). The method can resolve differences in diffusion coefficients of less than 50% (*5*). Translational diffusion coefficients of the proteins in native membranes estimated by FPR fall into three ranges. One range, $D < 1 \times 10^{-12}$ cm^2 s^{-1}, is characteristic of proteins that are immobile on the time scale (minutes) of an FPR measurement. Such proteins often form patches or clusters in the membrane; acetylcholine receptor of muscle is a good example. Translational diffusion of these proteins is probably constrained by their interaction with submembranous cytoskeleton and by self-aggregation (*2*). Other translational diffusion coefficients, notably that for vertebrate visual rhodopsin, are in the range $D > \sim 3 \times 10^{-9}$ cm^2 s^{-1}. Diffusion of these proteins is largely constrained by the viscosity of membrane bilayer lipids (*6*).

Translational diffusion of most membrane proteins falls in a range between these two extremes. *D* of the mobile fraction of such proteins is in the broad range 0.1×10^{-10} cm^2 s^{-1} to 10×10^{-10} cm^2 s^{-1} [values summarized in (*7*)]. We do not know how translational diffusion of these membrane proteins is constrained. There is some evidence that some of these proteins interact with proteins of the cytoskeleton. Extraction of cytoskeleton proteins with buffers or blebbing plasma membrane away from the underlying cytoplasm greatly increases translational diffusion coefficients of membrane proteins (*8*). However, translational diffusion of mutant membrane glycoproteins lacking almost all of their cytoplasmic domains does not differ significantly from that of the wild-type molecules (*9*). Translational diffusion of plasma membrane proteins anchored by inositol-lipids, rather than by peptides, is also constrained to nearly the same extent as that of conventional membrane proteins; *D* is in the range 5×10^{-10} cm^2 s^{-1} to 20×10^{-10} cm^2 s^{-1} (*10, 11*). We report that the translational diffusion of one membrane-integral glycoprotein, mouse H-2 L^d class I major histocompatibility complex (MHC) antigen, is largely constrained by the extent of glycosylation and by the size of its extracellular domains. Translational diffusion coefficients for mutants of L^d lacking oligosaccharides, or further truncated to approximately one-third the size of wild-type L^d, are up to a factor of 3 greater than diffusion coefficients for wild-type L^d. It appears that the external domains of L^d interact with other membrane proteins and with molecules in the medium bathing the cells to reduce translational diffusion of the protein.

The class I MHC antigens consist of three external domains (containing one to three sites for *N*-linked glycosylation), a transmembrane domain, and and a cytoplasmic domain. This chain is noncovalently associated with β_2-microglobulin (*12*). The mouse class I MHC antigen, H-2 L^d, bears three *N*-linked oligosaccharides. Miyazaki and coworkers (*13*) have prepared a set of mutants with one (C8.3), two (CM2), or all three (C3-1) glycosylation sites deleted from the external domains (Fig. 1). They showed that under- and unglycosylated L^d antigens were expressed in L cells transfected with the mutant genes. Other L^d mutants have been prepared that lack two of their three external domains (*14*). These mutants are also expressed in transfected L cells.

We used FPR to measure translational diffusion of wild-type and mutant L^d anti-

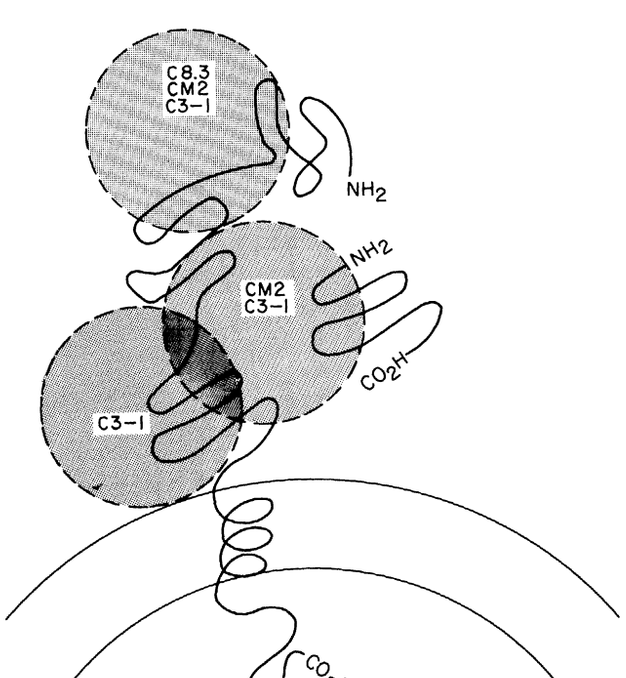

Fig. 1. A scaled cartoon of a class I MHC antigen [after (*12*)]. Recent crystallographic data give a molecular cross section of 50 Å by 40 Å and a total length of about 70 Å (*20*). The extent of the oligosaccharide units, from data in (*19*), is indicated by the hatched circles. The names of mutants lacking a particular oligosaccharide unit are listed within each circle.

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gens expressed in L cells as well as translational diffusion of the endogenous H-2 K^k antigens of these cells (*15, 16*). Translational diffusion coefficients of all of the mutant L^d antigens were larger than those of wild-type, fully glycosylated, L^d. The value for *D* was a factor of 3 greater than the value for wild type when all oligosaccharide units were lost (Fig. 2 and Table 1). An increase in *D*, to a value approaching that for viscosity-limited translational diffusion, was also found for L^d antigen lacking two of its three external domains (Table 2). The differences observed for *D* were not due to clonal variation in the transfected cells. Translational diffusion of endogenous K^k antigens varied by only about 20% between L-cell clones (Table 1).

A significant fraction of all L^d molecules was immobile in our experiments. Some of this immobile fraction may be an artifact of low levels of fluorescence. In our experience cell autofluorescence bleaches but does not recover. Hence the greater the contribution of autofluorescence to the total fluorescence the greater the underestimate of the mobile fraction of specific label. This is particularly true for clones C8.3 and C3-1, which express the mutant antigens poorly. However, immobile fractions are a common feature of FPR measurements even when labeling is well above autofluorescence or when recoveries are corrected for autofluorescence. These fractions are even found for inositol-lipid–anchored membrane proteins (*10, 11*). It appears that the mechanisms for immobilization of membrane proteins may differ from those affecting *D* of mobile molecules. The size of the immobile fraction may change independently of *D* when cell culture density increases or when cells differentiate (*17*). In some cells this is due to anchorage of a fraction of the population of labeled molecules to the cytoskeleton. It may also reflect the reorganization of cell surface domains (*18*).

The effect of glycosylation on *D* was only apparent when L^d antigens were labeled with Fab antibody fragments. L^d antigens labeled with intact immunoglobulin G (IgG) antibody all gave the same *D*, $\sim 5 \times 10^{-10}$ cm^2 s^{-1} regardless of the extent of their glycosylation (Fig. 2). The mobile fraction of L^d molecules labeled with IgG ranged from 35% ± 9% for C3-1 to 47% ± 14% for CM2. IgG labels did not alter translational diffusion of K^k antigens.

The branched oligosaccharide units of class I MHC antigens are larger than is suggested by the usual cartoon of these structures (*19*). The likely extent of each of the oligosaccharide relative to protein domains is indicated by shaded circles in Fig. 1. Both the bulk of the oligosaccharide units and their association (perhaps by hydrogen bonding) to other molecules, either in the surface membrane or in its neighborhood, would retard translational diffusion, particularly if some of the interacting molecules were immobile.

We suggest that the effects we have observed here could account generally for values of translational diffusion coefficients of membrane proteins in the range 10^{-11} to 10^{-9} cm^2 s^{-1}. The diffusion of these molecules would be hindered by their interaction with other anchored membrane proteins, with other mobile proteins, and with molecules external to the surface, including those on other cells. Variation in *D* for a given protein in different cells (*11, 16*) would then reflect variations in surface protein concentration, glycosylation, and extent of immobilization of all membrane proteins. Some of these variables, particularly membrane protein concentration, could affect translational diffusion in interior as well as in surface membranes.

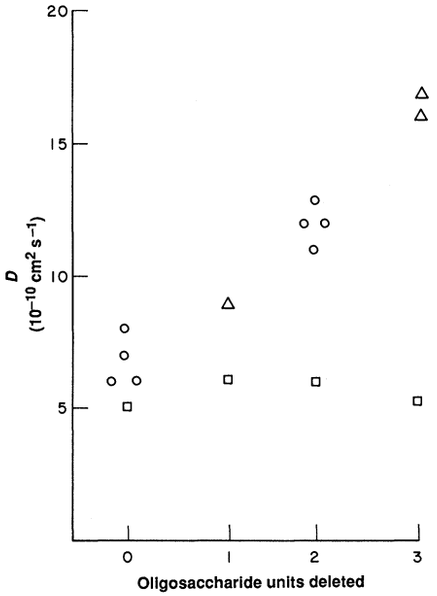

Fig. 2. Mean translational diffusion coefficients for wild-type and mutant L^d antigens. Each point represents one set of measurements on 10 to 20 cells expressing high (○) or low (△) levels of antigen. Cells were labeled with L^d-specific Fab fragments of an IgG MAb, except those indicated by □, which were labeled with the intact MAb (*15*).

Table 1. Lateral diffusion of wild-type and mutant L^d antigens.

Cell	L^d glycosylation sites	Expression	Antigen labeled	*D** (10^{-10} cm^2 s^{-1})	Mobile (%)	*n*
W12	3	High	L^d	6 (6–8)	37	84
C8.3	2	Low	L^d	9 (4–19)	32	21
CM2	1	High	L^d	15 (13–17)	39	81
C3-1	0	Low	L^d	17 (9–23)	39	22
W12	3	High	K^k	3.4 (3.3–3.6)	48	24
C8.3	2	Low	K^k	2.8 (2.5–3.1)	42	13
CM2	1	High	K^k	3.0 (2.7–3.2)	49	9
C3-1	0	Low	K^k	3.0 (2.1–4.2)	41	10

*Values are geometric mean and 95% confidence interval.

Table 2. Lateral diffusion of wild-type and truncated L^d antigens.

Cell	External domains of L^d antigen	Antigen labeled	D* (10^{-10} cm^2 s^{-1})	Mobile (%)	*n*
T.1.1.1	N, C1, C2	L^d	13 (13–14)	32	57
dmt9.10	C2	L^d	24 (24–25)†	39	95
dmt9.10	C2	K^k	2.2 (2.0–2.4)	31	12

*Geometric mean (95% confidence interval). †Differs significantly from value for T.1.1.1.

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15. Cells for photobleaching were plated onto glass cover slips 1 to 5 days before use. L^d antigens were labeled with fluorescein- or tetramethylrhodamine- (Molecular Probes, Eugene, OR) conjugated Fab fragments of the monoclonal antibody (MAb) 28-14-8, or with the fluorescein-conjugated IgG of the same MAb. Tetramethylrhodamine conjugates were purified over SM-2 BioBeads (Bio-Rad) to remove noncovalently bound rhodamine [E. Spack, Jr., *et al.*, *Anal. Biochem.* **158**, 233 (1986)]. The conjugates were centrifuged at 100,000*g* for 40 to 60 min before use. The conjugates labeled cells expressing L^d or the cross-reacting D^b MHC antigens and did not label L cells, which express H-2^k antigens, D^k and K^k. H-2^k antigens of L cells were labeled with fluorescein-conjugated Fab or IgG of MAb 11-4.1. Both labels gave the same *D* values. FPR measurements of labeled cells were made at 18° to 20°C with a 40× objective and a numerical aperture of 1.3, giving a spot radius (e^{-2}) of ~0.8 μm. Our computer-controlled photobleaching microscope has been described elsewhere (*16*).
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21. We thank K. Ozato for supplying the glycosylation-deficient mutants, J. McCluskey and D. Magulies for supplying the truncated L^d mutant, and T. Wei for technical assistance. Supported by NIH grant AI-14584 to M.E. M.W. was supported by an NIH training grant in immunology to K. Ishizaka. Contribution 1416 from the Department of Biology.

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A Graph-Dynamic Model of the Power Law of Practice and the Problem-Solving Fan-Effect

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Numerous human learning phenomena have been observed and captured by individual laws, but no unified theory of learning has succeeded in accounting for these observations. A theory and model are proposed that account for two of these phenomena: the power law of practice and the problem-solving fan-effect. The power law of practice states that the speed of performance of a task will improve as a power of the number of times that the task is performed. The power law resulting from two sorts of problem-solving changes, addition of operators to the problem-space graph and alterations in the decision procedure used to decide which operator to apply at a particular state, is empirically demonstrated. The model provides an analytic account for both of these sources of the power law. The model also predicts a problem-solving fan-effect, slowdown during practice caused by an increase in the difficulty of making useful decisions between possible paths, which is also found empirically.

THE POWER LAW OF PRACTICE (*1*), one of the few solid psychological learning phenomena, states that the speed of performance of a task will increase as a power of the number of times the task is performed. In one model, problem solving can be viewed as the search for a path through a directed "problem-space" graph, where nodes represent states of the problem or facts in memory and edges represent operators that move between states (*2*). Solving the problem involves finding a path from the initial state to the goal state by means of the available operators. Learning in this model corresponds to changes in either the specific topology of the graph or the decision procedure used to decide which operator to apply at a particular step when there is more than one edge emanating from a node. Many sorts of changes in method and operators can be modeled as changes in the topology of the problem-space graph, including restructuring and method selection. In this report we use computer experiments to show that this learning model exhibits the power law and that the phenomenon can be explained analytically by a theory based on graph dynamics. Our theory further predicts a problem-solving "fan-effect" in which performance becomes slower as more operators are learned in certain situations (*3*). This prediction is also empirically validated by our simulations.

The simple problem that we will use to explore learning phenomena, the "bit game," is analogous to many real problems. A problem state in the bit game is a *B*-bit binary vector (such as 01010). For the sake of concreteness we will use a 5-bit vector ($B = 5$) in most cases. A "trial" begins with an arbitrary initial state, say 00000. The player (a computer) searches for some other arbitrary vector (the goal state), say 11111, by successively applying operators that change the contents of the state vector. Each operator is composed of 1 to *B* elements indicating a particular bit in the vector that should be flipped if it matches in the current state. Operators specify only the bits in the state that actually change and can be written as "pattern → result" pairs, with question marks (?) where the operator pattern says nothing about a particular bit position. For instance, the operator ?1?1? → ?0?0? will take the state 11010 to 10000 or the state 11111 to 10101 but will not apply to the state 00000 because the bits indicated in the pattern do not match this state. As a result of the question mark "don't care" bits, operators vary in their generality. For instance, each of the two-element operators, such as 0?1?? → 1?0??, apply to eight different states (in this case 00100, 00101, 00110, 00111, 01100, 01101, 01110, and 01111).

We begin playing a particular bit game with all of the (2*B*) 1-bit operators (10, in the case of a 5-bit game). This set forms a *B*-dimensional hypercube and ensures that

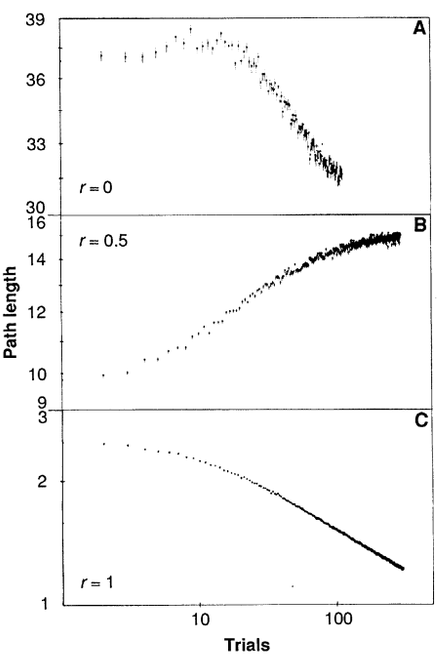

Fig. 1. Log-log plots of solution rates for the 5-bit game as a function of the number of trials: (**A**) the random walk; (**B**) mediocre decision procedure; (**C**) optimal decision procedure. All points are averaged over 16,384 observations.

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