particular dot but rather as a coherent wave that is delocalized over the two dots. The new state of the molecule as a whole is lower in energy than the states of the individual dots. This energy lowering is the binding force between the two dots. The experimental realization of two coherently coupled quantum dots would be very interesting because it has properties similar to those of a tunnel junction between two superconductors. In such two-level systems, radiation effects are very interesting. For instance, if the states in the two dots are not completely aligned, the energy difference may be overcome by the absorption and emission of photons from microwave radiation. This is expected to lead to new effects that are analogous to the ammonia molecule maser (6) or the alternating-current Josephson effect (7).

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Revisiting the Fluid Mosaic Model of Membranes

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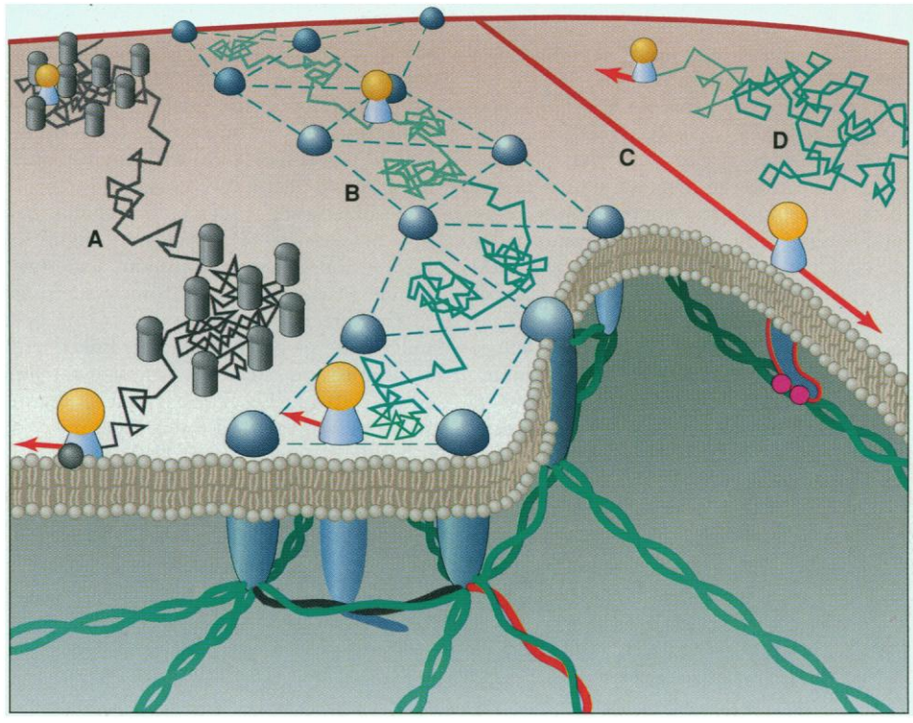
The fluid mosaic model, described over 20 years ago, characterized the cell membrane as "a two-dimensional oriented solution of integral proteins . . . in the viscous phospholipid bilayer" (*1*). This concept continues as the framework for thinking about the dynamic structure of biomembranes, but certain aspects now need revision. Most membrane proteins do not enjoy the continuous, unrestricted lateral diffusion characteristic of a random, two-dimensional fluid. Instead, proteins diffuse in a more complicated way that indicates considerable lateral heterogeneity in membrane structure, at least on a nanometer scale. Certain proteins are transiently confined to small domains in seemingly undifferentiated membrane regions. Another surprise is that a few membrane proteins undergo rapid, forward-directed transport toward the cell edge, perhaps propelled by cytoskeletal motors.

This more detailed view of the life of a membrane protein has emerged as a result of one old and two newer methods. For the past two decades, fluorescence recovery after photobleaching (FRAP) has been the major tool for measuring the lateral mobility of membrane components labeled directly with fluorophores or with fluorescent antibodies. In this method, a short pulse of intense laser light irreversibly destroys (photobleaches) the fluorophores in a micrometer-sized spot. The fluorescence gradually returns as fluorophores from the surrounding region diffuse into the irradiated area. FRAP experiments can reveal the fraction of labeled membrane proteins or lipids that can move, the rate of this movement (characterized by the lateral diffusion coefficient), and the fraction of proteins that cannot move on the time scale of the experiment. These apparently nondiffusing proteins are called the immobile fraction; a quantity that is frequently large and usually of unknown origin.

A second method, single-particle tracking (SPT), directly complements the information that is obtained from averaging the movement of hundreds to thousands of molecules in a FRAP experiment. In SPT, a membrane component is specifically labeled with an antibody-coated submicrometer colloidal gold or fluorescent particle, and the trajectory of the labeled molecule is followed with nanometer precision with digital imaging microscopy (*2*, *3*). Visualization of individual protein motions can reveal submicroscopic membrane structures as the protein encounters obstacles in its path, although careful data analysis is required to distinguish between nonrandom and random movements (*4*).

The third method, recently applied to membranes, is the optical laser trap, allowing further characterization of the obstacles a membrane protein encounters. Proteins are labeled with submicrometer beads and manipulated in the plane of the membrane with laser light. Optical trapping occurs when a near-infrared laser beam with a bell-shaped intensity profile is focused on the bead attached to the protein. Optical forces on the bead, which are directed toward the highest intensity of the beam, trap the particle (*5*). By moving the laser beam or the microscope stage, the labeled protein can



Lateral transport modes on the cell surface. (**A**) Transient confinement by obstacle clusters (**B**) or by the cytoskeleton, (**C**) directed motion, and (**D**) free random diffusion.

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be dragged across the plasma membrane until it encounters a barrier or obstacle that causes the bead to escape the trap. The distance between barriers is called the barrier-free path (BFP).

The fluid mosaic model proposes random, two-dimensional diffusion for membrane components. Although lipids (6) and a fraction of the labeled protein population appear by SPT to diffuse freely, other protein movements are considerably more complicated than originally envisioned in the fluid mosaic model. One big surprise has been that a substantial fraction of the proteins are confined, at least transiently, to small domains. This has been seen most clearly for certain cell adhesion molecules [cadherins and neural cell adhesion molecules (NCAMs)] and nutrient and growth factor receptors. For cadherins, transferrin receptors, and epidermal growth factor receptors, the domains are 300 to 600 nm in diameter and confinement lasts from 3 to 30 s (7). Following earlier work on the red cell membrane, Kusumi and colleagues (7) proposed the "membrane-skeleton fence" model. In this scheme, a spectrin-like meshwork closely apposed to the cytoplasmic face of the membrane sterically confines membrane-spanning proteins to regions on the order of the cytoskeletal mesh size. Support for this model includes the facts that partial destruction of the cytoskeleton decreases the fraction of confined molecules, and truncation of the cytoplasmic domain leads to less confined diffusion (8, 9).

Can the fence model be supported by other techniques? Enter the laser trap. In a pioneering study, Edidin *et al.* (10) showed that BFPs for the lipid-linked and the membrane-spanning isoforms of the major histocompatibility antigens were ~1700 and ~600 nm at 23°C, respectively, and that these values increased with the temperature, indicating the dynamic nature of the barriers. Using weaker trapping forces, Sako and Kusumi (11) could detect even smaller BFPs for the transferrin receptor (~400 nm), which are consistent with the size of domains measured by SPT for both this receptor and E-cadherin (7, 8). The fences appear elastic, because the transferrin receptor rebounds after it strikes barriers (11), and a small fraction of these receptors seem to be fixed to the underlying cytoskeleton by spring-like tethers (11).

To permit the long-range diffusion observed by both SPT and FRAP, these barriers must open temporarily, either by dissociation of key molecular constituents of the barriers or by thermally driven local fluctuations of the meshwork-membrane distance. The escape of a given protein into an adjacent domain probably depends on the size of its cytoplasmic moiety, which implies that the effective domain size may be protein-dependent (7, 12). The emerging picture is that the immobile fraction of membrane proteins measured by FRAP does not simply represent stationary proteins but rather is some combination of proteins actually tethered to the cytoskeleton and those moving within and between confinement zones.

Is direct trapping by the cortical cytoskeleton the only means of confinement? Probably not. Surprisingly, confinement was also found for a lipid-linked isoform of NCAM in muscle cells, which cannot be directly trapped by the cytoskeletal network. In this case, the membrane domains were ~280 nm in diameter, and the proteins remained in them for about 8 s (13). The confinement may be the result of interactions with the same or other proteins that are associated with the cytoskeleton. SPT analysis suggests that the proteins in these zones are diffusing through a dense field of obstacles. Presumably, such domains will transiently trap different proteins, although this has not been proven. Other glycosylphosphatidyl inositol–anchored proteins such as Thy-1 also exhibit tightly confined diffusion (14), possibly because they are sequestered in glycolipid-enriched regions that include caveolae (15). Such confinement zones could play a significant role in mediating adhesion or in signal transduction by collecting relevant molecules, for example, cell adhesion molecules with their cooperating growth factor receptors (16).

A diverse set of membrane proteins can also be seen with SPT to move by highly directed, nondiffusional transport, sometimes in unexpected directions. Some proteins go in the direction opposite that of the bulk movement of patches of cross-linked proteins into caps seen in lymphocytes and other cells (2, 17). For example, integrins move outward toward the cell periphery in a highly directed fashion (18). These cell-matrix adhesion receptors, which are important for cell locomotion, may be recycled from the back to the front of the cell by forward-directed cytoskeletal motors.

The plasma membrane presents an intriguing mix of dynamic activities in which components may randomly diffuse, be confined transiently to small domains, or experience highly directed movements (see figure). The coexistence of multiple modes of diffusion and directed transport indicates a pronounced lateral heterogeneity in the membrane. Key issues remain: How generally applicable is the membrane-skeleton fence model? Are transmembrane and glycosylphosphatidyl inositol–anchored proteins confined by the same structures in the cytoskeleton? How is the domain structure regulated? The greatest challenge will be to relate this exciting new knowledge of membrane dynamics to the manifold functions accomplished by the plasma membrane.

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