windows on cosmological events and processes.

There is a clear experimental route toward elucidating the processes of baryosynthesis, as was described in the last section. In the next decade, experiments on proton decay and the neutron electric dipole moment (see Fig. 4, a and b) will either provide confirmation of the scheme sketched here or insights into new theories.

The horizon and flatness problems are certainly not yet solved. Examining the large-scale structure of the Universe by using large telescopes on the ground, the Space Telescope, and the Cosmic Background Explorer satellite (see Fig. 4, c and d) will help to elucidate the origin and evolution of the deviations from a homogeneous, flat cosmology. It will also shed light on the current value of the expansion rate, help to determine whether the Universe is open or closed, and place better limits on the current energy density of the vacuum.

An important problem in need of solution by theorists is the current tiny value of the vacuum energy density (the cosmological constant) relative to that expected in our current low-temperature vacuum state. The solution may involve a new, fundamental principle that is certain to have a broader impact than the resolution of the cosmological problems discussed here.

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Endocytosis: Relation to Capping and Cell Locomotion

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In this article, I draw together several aspects of the endocytic cycle initiated by coated pits in the cell's plasma membrane (*1–4*). I suggest that in motile cells this cycle causes a bulk flow of particular membrane components across the cell's surface. A natural consequence of this flow is that any large aggregate on the cell's surface will be swept to the rear of the cell. This movement of aggregates, which is known as capping, depends on the fluid nature of membranes. Finally, I suggest how the endocytic cycle may be part of the machinery a cell uses in locomotion, and what this implies for the difference between motile and nonmotile cells.

The Endocytic Cycle

The function of coated pits in adsorptive endocytosis was first deduced from studies on developing mosquito oocytes (*5*). In thin sections viewed by electron microscopy, coated pits appear as depressions or invaginations in the cell's surface. A coated pit is distinguished from other depressions by its characteristic, thick (about 200 Å) coat at its cytoplasmic surface. Coated pits on oocytes usually bear many yolk particles attached to their outer surfaces; in the same sections coated vesicles that bear yolk particles are seen inside the cell. Therefore, coated pits presumably bud into a cell to yield coated vesicles, and their function in oocytes is to bring yolk particles into the cell to form the yolk (*5*).

Coated pits have now been observed on the surface of almost all cells, except erythrocytes (*1, 6*). These pits serve to bring specific macromolecules into the cell, but which macromolecule is determined by the specific receptors present in the coated pit; and the specific receptors, in turn, depend on the cell type. Thus, oocytes have receptors for yolk proteins, which bring the yolk into those cells. Infant rat gut epithelial cells have immunoglobulin G receptors that bind antibodies from the mother's milk; the antibodies are internalized by coated pits and eventually they are transferred across the epithelium to the infant's blood circulation, a mechanism that provides the infant rat with passive immunity during its early life (*7*).

The properties of coated pits and their associated receptors have been studied most extensively in cells grown in culture. The best understood receptor is that for low density lipoprotein (LDL) (*8*). The LDL is a large particle originating in the liver that circulates in the plasma; it is taken up by many cells and degraded, and the cholesterol so liberated serves as the main source of cellular cholesterol. When fibroblasts in culture are starved of LDL, the number of LDL receptors is increased to about 10,000 per cell.

The distribution of these receptors on the cell surface has been determined by adding ferritin-conjugated LDL to human fibroblasts at 4°C (at this temperature the cells bind LDL, but do not undergo endocytosis). When thin sections of such labeled cells are examined, about two-thirds of the LDL-ferritin is found in coated pits; nevertheless, coated pits account for only about 2 percent of the cell's surface (*9*). If cells labeled with LDL-ferritin at 4°C are warmed to 37°C for a few minutes, much of the ferritin is found in coated vesicles or in smooth vesicles inside the cell. At later times (30 minutes at 37°C) the LDL is found in lysozomes. The LDL receptor is returned to the cell surface to be reutilized for many further cycles since the uptake of LDL is unaffected by the presence of inhibitors of protein synthesis over a period of several hours (*2*). Such experiments (*5, 8*) indicate that the

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sequence of events is (i) LDL binds to its receptor in a coated pit, (ii) the pit buds into the cell yielding a coated vesicle, (iii) the vesicle sheds its coat, the LDL is released from its receptor, and several vesicles fuse together, (iv) the LDL receptor is returned to the cell surface to enter another endocytic cycle, and (v) the LDL is transported to lysozomes where it is degraded.

Summary. Most mammalian cells, such as fibroblasts, continuously internalize part of their surface membrane by endocytosis, and then later return it to the cell surface. This cyclical process is initiated by coated pits in the plasma membrane. These pits collect specific receptors plus lipid for internalization, but exclude other proteins. On a motile cell, the sites of endocytosis (randomly located on the cell) and those of membrane return (located at the front of the cell) are not coincident. This causes a bulk flow of lipid plus receptors in the plasma membrane, away from the front of the cell. Large objects on the cell surface are swept to the rear of the cell by this flow, a process called capping. Cells may use this polarized endocytic cycle to move.

Although most of the LDL receptors on the surface of human fibroblasts are located in coated pits at any moment, there is considerable variation in the proportion of receptors bound to coated pits. This proportion depends on the cell type and the receptor. For example, when fully induced to produce LDL receptors, the human carcinoma A-431 line has about 2×10^5 LDL receptors per cell, of which only about 4 percent are located in coated pits (*10*). On HeLa cells, the surface ferritin receptors are essentially all localized in coated pits (*11*), whereas only about 10 percent of the transferrin receptors on the same cells are in coated pits (*12*).

Coated pits as molecular filters. Coated pits on fibroblasts contain not just LDL receptors, but a host of other receptors. These include the receptors for transferrin, transcobalamin, epidermal growth factor, α_2 macroglobulin, and proteins carrying mannose 6-phosphate residues (*13*). Each coated pit is roughly 0.1 μm^2 in area and may contain about 1000 receptors; each pit contains several different kinds of receptors.

Because coated pits are packed with receptors, the concentration of other plasma membrane proteins in them may be diminished. In a study to test this possibility, fibroblasts were labeled at 0°C with antibodies directed against a surface protein (*14*), and these antibodies themselves were detected with a ferritin-conjugate to the antibody. The concentration of ferritin particles in the membrane of coated pits was compared with that along the rest of the plasma membrane. This showed that, for two major surface proteins on fibroblasts, their concentrations in coated pits were 1 percent or less of that along the rest of the plasma membrane. In other words, these two surface proteins are essentially excluded from coated pits. Coated pits, then, act as molecular filters; they collect specific receptors embedded in the lipid bilayer into a small domain, and exclude the rest of the surrounding membrane proteins (*3, 14*).

How coated pits are able to exclude other proteins is unclear, but a knowledge of the structure of coated pits or coated vesicles should provide some clues. Thus, it seems likely that the cytoplasmic coat, made up of a protein called clathrin (*15*), enables specific receptors to bind to a coated pit in such a way that there is simply no space left for other, nonbound, membrane proteins. In any event, coated pits on the surface of a cell seem to bud in specific receptors plus lipid, but not the other plasma membrane proteins (*16*).

Rate of endocytosis. The rate at which coated pits internalize has been estimated, as described above, in several different systems including the uptake of LDL by human fibroblasts (*17*), the uptake of Semliki Forest virus by baby hamster kidney (BHK) cells (*18*), and the uptake of asialoglycoproteins by hepatocytes (*19*). It is difficult to obtain an accurate value for the lifetime of a coated pit, but the evidence suggests that it is about a minute—perhaps a bit less. Because coated pits on fibroblasts (*8*), giant HeLa cells (*11*), and BHK cells (*18*) account for about 2 percent of the cell surface, these cells must take up the equivalent of their surface areas each 50 minutes or so. As coated pits are about randomly distributed on the surfaces of human fibroblasts (*17, 20*) and giant HeLa cells (*11*), it is reasonable to suppose that surface uptake, over a period of time, occurs uniformly over the cells' surfaces.

Receptor transit times. Most receptors, once internalized, are recycled and returned to the cell's surface. How long the receptors remain inside the cell in each endocytic cycle—their transit times—can be estimated from the time taken for a surface complement of receptors to undergo endocytosis (the surface residence time), and the relative proportions of the receptor on the surface compared to that inside the cell. If all the receptors are on the same cycling route, the transit time is provided by the product of the surface residence time and the ratio of receptors inside the cell to those outside. Thus, for the transferrin receptor on HeLa cells, a full complement of receptors (about 10^5 molecules) undergoes endocytosis every 7 minutes, and there are about three times as many receptors in transit inside the cell as there are on the surface. This receptor, on these cells, therefore has a transit time of about 21 minutes (*21*). Different receptors may have different transit times; the LDL receptor on human fibroblasts has a very short transit time—less than 1/4 minute since there is no detectable pool of this receptor inside the cells (*22*).

Return of the receptor to the cell surface. The exact route taken through the cell by any receptor is unknown. Once internalized, the LDL receptor parts company with the LDL it has brought into the cell; the receptor rapidly returns to the cell surface, and the LDL is transported to lysozomes where it is degraded. The transferrin receptor in HeLa cells and probably also in mouse teratocarcinoma cells (*23*) has a much longer transit time. It takes transferrin through the cell; during this transit, the transferrin probably remains bound to its receptor, releasing bound iron in a low *p*H compartment inside the cell. This is the principal source of iron for most cells.

But to where on the cell surface the internalized membrane is returned has not been directly determined. However, two different answers seem to be available; what happens depends on the state of the cell. In the first case, exemplified by a stationary human fibroblast (*24*), the membrane to be internalized is taken up uniformly over the cell's surface: it is probably returned to the cell surface nearby because the transit time for the LDL receptor is measured in seconds rather than minutes, and it is unlikely that membrane vesicles can be moved very far in that time. This picture is consistent with the roughly uniform distribution of LDL receptors seen on stationary fibroblasts. The second case is illustrated by giant HeLa cells, where the returned membrane is not added to the cell surface uniformly. Giant HeLa cells are nondividing cells that grow to enormous sizes: they are substrate-attached cells that look roughly like a fried ("sunny-side up") egg, and have diameters as large as 500 μm (*25, 26*). On these

cells, coated pits are randomly distributed; however, the LDL, transferrin, and ferritin receptors are concentrated toward the cell's periphery, having a lower surface density in the middle of the cell (*11, 27*). The interpretation of this observation is that these receptors are returned to the cell surface at the cell's periphery, or leading edge, and that they are internalized and returned to the leading edge before they can diffuse to the middle of the cell. In a sense, they are trapped (*28*). Significantly, an earlier study revealed that a newly synthesized protein, on these same cells, is also added to the cell surface at its leading edge (*26, 29*).

I think the conclusion to be drawn here is that on some cells, such as stationary fibroblasts, the membrane that has undergone endocytosis is returned to the cell surface randomly; on others, such as giant HeLa cells, it is added to the cell's leading edge. This difference is discussed below. For the present, I now focus on those cells whose membranes, like that of the giant HeLa cell, are returned to the surface at their leading edges (*30*).

Capping of Surface Antigens

In the endocytic cycle mediated by coated pits, the cells internalize specific receptors plus lipid, but not other surface proteins, at an enormous rate. This uptake occurs over the entire cell, but is returned to the cell's surface at a particular region. Because the sites of endocytosis and return are not coincident, there is a net flow along the plasma membrane from the sites of exocytosis toward the sites for endocytosis. This flow is made up of lipid plus receptors. This bulk flow of membrane components has an interesting effect that can best be understood after consideration of what happens to a membrane protein that does not participate in this endocytic cycle (i) when it is not cross-linked and (ii) when it has been cross-linked by a specific antibody to form a large aggregate, or patch. Since the matrix in which these proteins lie is a lipid bilayer, it is simplest to think of the flow as a lipid flow (and disregard here the fact that there is also a receptor flow) (*31*).

Figure 1 provides an example of a motile fibroblast in which the returned membrane is added at the cell's leading edge and uptake occurs all over the cell. A noncycling protein in the plasma membrane will tend to be swept along with the flow toward the rear of the cell; in contrast, this protein can also diffuse about by Brownian motion, and therefore randomize its distribution. Which process prevails depends on how fast the flow is, how fast the protein can diffuse (defined by its diffusion coefficient, D), and how long the cell is. Calculation (*31, 32*) shows that if the flow is 1 μm/min (*33*), if the cell is 20 μm long, and if D is 10^{-8} cm^2/sec, then at equilibrium the extent to which the protein gets swept along by the flow, given by the ratio of the protein's concentration at the leading edge compared to that at the tail of the cell, is 0.7. In other words, the protein does get swept away from the leading edge a bit, but not by much. Diffusion has more or less won (*31, 34, 35*).

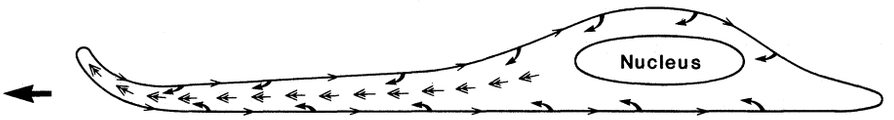

Fig. 1. Schematic diagram of a cross-section through a motile fibroblast moving toward the left (←). Endocytosis of lipid plus receptors is by coated pits (↶); this membrane that has undergone endocytosis is transported through the cell (≪—) and reinserted at the cell's leading edge. This causes a net flow (—≫) of these membrane components along the cell's plasma membrane.

But what happens if the protein is cross-linked with an antibody, so that 10^4 copies of this protein are joined into a patch? The diffusion coefficient of the aggregate would be decreased—perhaps to as little as 10^{-10} cm^2/sec. Now calculation shows that, at equilibrium, the ratio of the concentration of this aggregate at the leading edge compared to that at the tail of the cell (in a uniform flow of 1 μm/min) would be about 10^{-14} (*35*). In other words, all the aggregates would have been swept along by the flow to the tail of the cell; flow would win and the patch would have "capped."

The phenomenon of capping was discovered in 1971 (*36, 37*). If a surface antigen is cross-linked extensively with antibodies at 0°C, the antigens form a patch. This is simply a small two-dimensional precipitate of the antigen-antibody complex on the cell surface. However, if the cell is motile, the patches move to one end of the cell when it is warmed up to 37°C. This local concentration of the antigen toward the tail of the cell is called a cap (*36*).

The process of capping—the migration of patches to one end of the cell—has been studied extensively (*38, 39*). (i) It requires metabolic energy, as does the endocytic cycle. (ii) It occurs only if the cells are kept warm; below about 15°C it stops (*39*), as does endocytosis (*40*). (iii) It is a polar process in that caps form at a region remote from the leading edge of lymphocytes (*36*), motile fibroblasts (*41, 42*), giant HeLa cells (*26*), or epithelial cells (*43*), as would be expected if patches were swept away from the leading edge by a flowing membrane. But potentially motile cells do not need to be attached to a substrate to cap; B lymphocytes in suspension were the first cells shown to cap, and do so at the tail of the cell (*36*). (iv) Only those surface proteins that are extensively cross-linked cap; the others remain unaffected (*36*). And essentially any protein suitably cross-linked, whether with antibodies or other agents, caps (*36, 39, 44*). This shows that capping is a general property of aggregates on the cell's plasma membrane, and not a peculiarity of certain proteins. A general mechanism is thus required to explain it, such as membrane flow.

Several experimenters have studied the effects of the drugs cytochalasin B (which is believed to inhibit the action of microfilaments) and colchicine (which disrupts microtubules) on capping. Although the results vary, colchicine usually has little effect on its own, whereas cytochalasin B has a definite but not profound effect (about a third of the cells still cap). However, in combination, these two drugs eliminate capping. This has led to a widespread conception that microtubules or microfilaments (or both) take part in the physical movement of patches to form caps (*45*). Perhaps the best evidence that this may not be so are the observations that glycolipids and Thy-1 antigen (*46*)—neither of which have domains on the cytoplasmic side of the membrane—cap when suitably cross-linked (*36, 47, 48*). In these cases no part of the cytoskeleton can interact directly with a patch as it caps.

A different line of evidence—that parts of the cytoskeleton may be involved in capping—comes from studies of patch and cap formation on lymphocytes, monitored by immunofluorescence. When, for example, surface immunoglobulin on B cells is cross-linked by an appropriate antibody in the cold, the induced patches are seen to be associated with "subpatches" of actin. When the cells are warmed, both patches and subpatches cap (*49*). This apparent association between caps and "subcaps" is taken as evidence that actin is the driving force

for cap formation. That this is an unconvincing argument can be seen by considering patch formation; were the mechanism of patching unknown, the formation of actin subpatches could be interpreted as evidence that actin also drives patch formation. Of course, this view does not prevail. How actin subpatches form is unknown, but it is possible that the cross-linking antibody molecules cross-link surface molecules on adjacent microvilli on the surface of these cells, thereby clumping the microvilli. Since these villi contain substantial amounts of both actin and surface membrane (and hence cross-linked surface immunoglobulin), subpatches might be seen by immunofluorescence. If this explanation were correct, the two sets of molecules would cap togther.

It has been known for some time that different antigens cap at different rates (*50*). For example, surface immunoglobulin on B lymphocytes caps very quickly (in a matter of minutes), whereas histocompatibility antigens do so more slowly on the same cells (requiring perhaps 15 to 30 minutes at 37°C). This is naturally explained by supposing that the rate of capping depends on the size of the patch; a small patch would have to diffuse around and amalgamate with others to form large patches before it could cap efficiently (*51, 52*).

The properties of the endocytic cycle on motile cells can, then, readily explain the phenomenon of capping: large patches flow away from the region of membrane exocytosis in a passive fashion, whereas individual proteins overcome this flow by Brownian motion. For this to occur, individual proteins must have diffusion coefficients (D) of about 10^{-8} cm^2/sec at 37°C (*53*). The diffusion coefficients of many membrane proteins have been measured—the first was that of rhodopsin (3×10^{-9} cm^2/sec at 22°C, which would give a value of $\sim 10^{-8}$ cm^2/sec at 37°C) (*54*). Three other independent methods on different proteins have given about the same result (*55, 56*). However, a fifth method, fluorescence photobleaching recovery (FPR), which has been extensively used, often yields much lower D values. For example, the unlocalized acetylcholine receptor on myoblasts has a D of 5×10^{-11} cm^2/sec at 22°C when measured by FPR (*57*), although by a different method its value is 2.6×10^{-9} cm^2/sec at 22°C (*56*). It is clear that at present the true value of diffusion coefficients remains unresolved (*58, 59*), although most methods provide values equivalent to $\sim 10^{-8}$ cm^2/sec at 37°C.

Cell Locomotion

Too little is known about how cells move over a substratum to be certain whether a cell has just one mode of locomotion or several, and whether different kinds of cells use different mechanisms (*60, 61*). However, it is a feature of almost all locomotory mammalian cells that they cap cross-linked surface antigens, whereas nonmotile cells do not. This association between the abilities to migrate and to cap surface antigens implies that the two processes may be related. Here I should like to indicate how a membrane flow scheme, as shown in Fig. 1, could be used by a cell to effect locomotion. In that the migrating behavior of fibroblasts has been studied in greatest detail, I now focus on them; but it is likely that many other cells move similarly.

One of the most revealing experiments on cell locomotion dealt with the behavior of fibroblasts migrating from a tissue explant by time-lapse photography (*62, 63*). The cells were migrating on a cover slip to which small carbon particles adhered. When the leading edge of the fibroblast encountered a particle, the latter sometimes became attached to the dorsal (upper) surface of the cell. When it did so, the particle migrated away from the leading edge on the cell surface at a roughly uniform rate (about 2 μm/min) and in approximately a straight line (*62, 64, 65*). Eventually the particle came to rest toward the rear of the cell. This experiment suggests that, since the particle is attached to the membrane and is moving, the membrane is moving (*62*). As a result it was proposed that the fibroblast takes up its surface membrane at the rear of the cell (where the particles accumulate) and reinserts this membrane at the cell's leading edge (*62*). This would provide a mechanism whereby the cell can extend itself ahead of itself. This proposal was the first concise description of how a fibroblast extends itself (*66*): its relationship to the endocytic cycle described above should be clear. Thus, the endocytic cycle of a motile cell can be used to extend the leading edge of that cell forward.

The leading edge of a fibroblast is a site of tremendous membrane activity; it ruffles and puts out protrusions there. This is all consistent with its being the site at which large amounts of internal membrane are added to the cell surface (*67*). In the carbon particle experiment, the initial rate of rearward migration of particles was about 2 μm/min. As these cells are roughly triangular (about 100 μm long), this would require the cells to take up their entire surfaces (dorsal plus ventral) every 25 minutes (*34*). The actual rate of surface uptake may not be as high as this since not all regions of the leading edge are equally active in ruffling at any given moment. The main point here is, however, that the rate of rearward particle migration, which is also the rate at which large patches move rearward on these cells (*42*), requires a rate of surface uptake similar to that actually found to be taken up by coated pits on fibroblasts and BHK cells in culture, as mentioned earlier.

The picture, then, is that a fibroblast extends forward by inserting the internalized membrane into its leading edge. As soon as this membrane (composed of circulating receptors plus lipid) has been added to the plasma membrane, diffusion mixes it with noncirculating plasma membrane components so that it more closely resembles the average plasma membrane. But this circulating membrane could be used not only to extend the front of the cell but also to move the whole cell forward. Consider a cell migrating in vivo over a substratum (which might, for example, be a basement membrane). Presumably the cell interacts with this substratum through plasma membrane proteins that bind weakly to it; these weak bonds would be continually being made and broken. If the interacting proteins belong to the circulating receptor class, the following situation would exist. While the receptor is attached to the substrate, the force that propels the receptor backward also would tend to push the cell forward. However, once an interaction came undone, the receptor involved could again undergo endocytosis and be brought to the cell's leading edge to be reused. The cell would move forward, rather like a tank does on its track.

Some cells in culture (and presumably many cells in vivo) are subject to contact inhibition of movement (*68*). When two cells meet, the sites of contact stop ruffling and cell extension at those contact regions ceases (*69*). How this occurs is unknown, but it seems likely that this contact inhibition is achieved by a cessation of exocytosis of circulating membrane at those points of contact. This leads to two unresolved, but related, questions: how an oriented cell directs its circulating membrane to exocytose at just the cell's leading edge, and to where on the cell surface does a nonmotile cell (such as a stationary, spread fibroblast) return its circulating membrane. It is possible that on a stationary cell the

internalized membrane is returned at random to the cell surface, whereas on a motile cell it is transported through the cell to its leading edge. One might therefore expect that the transit time for the passage of receptors through a cell would be greater for motile cells than for stationary ones (*70*). How a motile cell achieves the addition of circulating membrane to a restricted region of the plasma membrane—to form the ruffling edge—must depend on how the cytoplasm is organized; key elements in this organization are likely to include components of the cell's cytoskeleton.

Conclusion

The endocytic cycle initiated by coated pits on the surfaces of motile mammalian cells causes a net flow of lipid plus specific receptors from the leading edge of the cell in a rearward direction. This flow tends to sweep noncirculating proteins toward the cell tail, but their distribution is kept roughly random by Brownian motion. However, large aggregates will be swept backward and cap.

This flow is used by motile cells to extend themselves forward during cell locomotion, and can perhaps serve to move the entire cell forward. The mechanisms inside the cell that direct the circulating membrane thereby determine whether the cell should move or remain stationary, and if it does move, in which direction it will do so.

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26. P. I. Marcus, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 351 (1962).
27. M. S. Bretscher, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 454 (1983).
28. Polymorphonuclear leukocytes move by chemotaxis toward certain peptides. This peptide receptor is concentrated toward the leading (anterior) end of the cell [S. J. Sullivan and S. H. Zigmond, *J. Cell Biol.* **95**, Abstract 21029 (1982)], possibly enabling the cell to sense a concentration gradient. In addition, transferrin receptors seem to be concentrated toward the leading edge of motile fibroblasts [P. Ekblom, I. Thesleff, V-P. Lehto, I. Virtanen, *Int. J. Cancer* **31**, 111 (1983)]. These receptor gradients might be analogous to those described on giant HeLa cells (*11, 27*).
29. These observations suggest that recycled receptors and newly synthesized membrane proteins may be transferred together inside the cell to the cell's leading edge. A recent report indicates that newly synthesized proteins may be added to the leading edge of motile fibroblasts [J. E. Bergmann, A. Kupfer, S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1367 (1983)].
30. Giant HeLa cells are normally round and do not move. However, they are motile cells in the sense that a macroscopic object (such as an erythrocyte), when attached at the cell periphery, is moved toward the center of the cell where it forms a cap (*26*). This movement is analogous to the migration of carbon particles on fibroblasts (*62*) and indicates that a triangular motile fibroblast is equivalent in this sense to a secant of a giant HeLa cell. Giant HeLa cells do not move because they are presumably "trying" to move in every direction at once. Their leading edges continually ruffle.
31. M. S. Bretscher, *Nature (London)* **260**, 21 (1976).
32. If protein molecules (having a diffusion coefficient *D*) reside in a uniformly flowing matrix (of flow rate *F*), then the concentration ratio at two points A,B (where A is upstream of B by length *L*) is given by $C_A/C_B = e^{-FL/D}$ (*31*).
33. This is the approximate rate at which objects on the surface of fibroblasts (*62*) or giant HeLa cells (*26*) are swept backward.
34. M. S. Bretscher, *Cold Spring Harbor Symp. Quant. Biol.* **46**, 707 (1982).
35. The situation is more complex than is stated here. The original proposal (*31*) on lipid flow was based on the premise that endocytosis and exocytosis occurred at restricted sites [as was also proposed earlier (*62*)]. We now know that, while the site of exocytosis is localized on a motile cell, the sites of endocytosis are not. This means that the rearward flow rate is not uniform, as assumed here, but decreases from the leading edge. Thus, any gradient of molecules that are not cross-linked would be shallower than that indicated, as would be that of cross-linked ones. In fact, there would be no bulk flow at the tail of the cell; aggregates would be swept rearward from the leading edge to a region on the cell behind which diffusion can overcome the flow. This implies that aggregates of different sizes would be swept rearward to different extents. For calculations with a decreasing flow rate, see (*34*).
36. R. B. Taylor, W. P. H. Duffus, M. C. Raff, S. de Petris, *Nature (London) New Biol.* **233**, 225 (1971); S. de Petris and M. C. Raff, *Eur. J. Immunol.* **2**, 523 (1972); *Nature (London) New Biol.* **241**, 257 (1973).
37. For similar phenomena see (*26, 62*) and B. M. Shaffer [*Exp. Cell Res.* **32**, 603 (1963)].
38. For almost every "fact" about capping, there is an "anti-fact." The main properties listed here would probably be agreed to by most workers in the field.
39. See the very extensive review by S. de Petris, in *Methods Membr. Biol.* **9**, 1 (1978).
40. J. L. Goldstein, in (*1*), p. 28.
41. M. Edidin and A. Weiss, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2456 (1972).
42. M. Abercrombie, J. E. M. Heaysman, S. M. Pegrum, *Exp. Cell Res.* **73**, 536 (1972).
43. J. M. Vasiliev, I. M. Gelfand, L. V. Domnina, N. A. Dorfman, O. Y. Pletyushkina, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4085 (1976). These investigators showed that when epithelial cells cap concanavalin A receptors, the receptors are moved away from free edges.
44. K.-G. Sundqvist, *Nature (London) New Biol.* **239**, 147 (1972).
45. That both drugs are needed to eliminate capping is sometimes considered as evidence that microfilaments and microtubules cooperate in the process. However, if they did cooperate, either drug alone should stop cap formation. That both drugs are needed implies their effects cannot be simply interpreted [see (*67*)]. For arguments in favor of cytoskeletal mechanisms for capping, see (*39*).
46. D. G. Campbell, J. Gagnon, K. B. M. Reid, A. F. Williams, *Biochem. J.* **195**, 15 (1981).
47. S. W. Craig and P. Cuatracasas, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3844 (1975); T. Revesz and M. Greaves, *Nature (London)* **257**, 103 (1975).
48. P. L. Stern and M. S. Bretscher, *J. Cell Biol.* **82**, 829 (1979).
49. L. Y. W. Bourguignon and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5031 (1977). More recent papers from other laboratories have reported similar results for other components of the cytoskeleton.
50. See, for example, the study by J. Braun, K. Fujiwara, T. D. Pollard, E. R. Unanue, *J. Cell Biol.* **79**, 409 (1978).
51. The relation between patch size and diffusion coefficient (*D*) is complicated to calculate. An equation that relates *D* to protein size has been derived [P. G. Saffman and M. Delbrück, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3111 (1975)], but this does not hold for aggregates whose diameter is greater than a few hundred angstroms. For a very large patch, the viscous drag of the bilayer no longer limits diffusion; the patch is equivalent to a flat disk moving through a medium of viscosity determined by the external medium and cytoplasm. If these viscosities (μ) are equal, then

$$D = \frac{3kT}{32\,\mu a}$$

[where T is the absolute temperature, k is Boltzmann's constant, and a is the radius of the disk; see H. Lamb, in *Hydrodynamics* (Cambridge Univ. Press, London, 1932]. For a 10-μm disk in water, $D \approx 3 \times 10^{-10}$ cm^2/sec. Of course, this is not meant to imply that a large patch on a cell would have that *D*, as the viscosity of the cytoplasm on that scale is unknown.
52. Coated pits themselves might get partially capped on macrophages [J. R. Pfeiffer, J. M. Oliver, R. D. Berlin, *Nature (London)* **286**, 727 (1980)].
53. If the diffusion coefficients of surface proteins were as low as 10^{-10} cm^2/sec, they would be unable to diffuse against the proposed flow, and so would cap, even when they are not cross-linked.
54. M-m. Poo and R. A. Cone, *Nature (London)* **247**, 438 (1974).
55. M. Edidin and D. Fambrough, *J. Cell Biol.* **57**, 27 (1973); M-m. Poo, W-j. H. Poo, J. Lam, *ibid.* **76**, 483 (1978); M-m. Poo, J. Lam, N. Orida, A. W. Chao, *Biophys. J.* **26**, 1 (1979).
56. M-m. Poo, *Nature (London)* **295**, 332 (1982).

57. D. Axelrod *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4594 (1976).
58. R. J. Cherry [*Biochim. Biophys. Acta* **559**, 289 (1979)] reviews this topic.
59. See also C-L. Wey, R. A. Cone, M. A. Edidin, *Biophys. J.* **33**, 225 (1981); D. E. Wolf, M. Edidin, P. R. Dragsten, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2043 (1980).
60. R. Porter and D. W. Fitzsimons, Eds., *Locomotion of Tissue Cells* (Elsevier, Amsterdam, 1973).
61. For perspectives different from that offered here, see J. P. Trinkaus, *Prog. Clin. Biol. Res.* **41**, 887 (1980); G. Albrecht-Buehler, *Cold Spring Harbor Symp. Quant. Biol.* **46**, 45 (1982).
62. M. Abercrombie, J. E. M. Heaysman, S. M. Pegrum, *Exp. Cell Res.* **59**, 393 (1970); *ibid.* **62**, 389 (1970).
63. V. M. Ingram, *Nature (London)* **222**, 641 (1969).
64. See also A. K. Harris, in (*60*), pp. 3–20.
65. A. Harris and G. Dunn, *Exp. Cell Res.* **73**, 519 (1972). In addition, it was shown that particles that are free to move on the ventral surface of the cell behave similarly.
66. Earlier proposals of a related nature were made by R. J. Goldacre, *Exp. Cell Res. Suppl.* **8**, 1 (1961); B. M. Shaffer, *Exp. Cell Res.* **37**, 12 (1965); *J. Theor. Biol.* **8**, 27 (1965).
67. The effects of drugs that affect the cytoskeleton are interesting: cytochalasin B inhibits the ruffling membrane of fibroblasts [S. B. Carter, *Nature (London)* **213**, 261 (1967)] and nerve growth cones [K. M. Yamada, B. S. Spooner, N. K. Wessells, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1206 (1970)], whereas colchicine has no obvious effect [B. S. Spooner, K. Yamada, N. K. Wessells, *J. Cell Biol.* **49**, 595 (1971)]. Colchicine treatment does, however, lead to a loss of directed movement in fibroblasts, although the locomotory system still functions [J. M. Vasiliev *et al.*, *J. Embryol. Exp. Morphol.* **24**, 625 (1970)]. The effects of these drugs on the capping process agree well with their effects on ruffling, further suggesting that the two processes are related.
68. M. Abercrombie and J. E. M. Heaysman, *Exp. Cell Res.* **6**, 293 (1954).
69. M. Abercrombie and E. J. Ambrose, *ibid.* **15**, 332 (1958).
70. The transit time for the low-density lipoprotein (LDL) receptor on stationary human fibroblasts is very short—a fraction of a minute. I expect that the transit time for this receptor on motile cells would be greater, as would be the proportion of these receptors inside the cell. If this is so, it would tie cell motility to a biochemical measurement.
71. I thank my colleagues for helpful suggestions, especially G. Mitchison, F. H. C. Crick, R. D. Kornberg, B. Pearse, and H. C. Berg.

Replication Timing of Genes and Middle Repetitive Sequences

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Summary. DNA replication in mammals is temporally bimodal. "Housekeeping" genes, which are active in all cells, replicate during the first half of the S phase of cell growth. Tissue-specific genes replicate early in those cells in which they are potentially expressed, and they usually replicate late in tissues in which they are not expressed. Replication during the first half of the S phase is, therefore, a necessary but not sufficient condition for gene transcription. A change in the replication timing of a tissue-specific gene appears to reflect the commitment of that gene to transcriptional competence or to quiescence during ontogeny. Most families of middle repetitive sequences replicate either early or late. These data are consistent with a model in which two functionally distinct genomes coexist in the nucleus.

The biphasic nature of DNA replication in eukaryotic cells (*1*) allows us to speak of two classes of replication units or "replicons" (*2*), early and late (Fig. 1A). The replicons in each temporal class are found in clusters of about 20 (*2*), and these can be resolved into a longitudinal pattern of early and late replication bands by substitution with bromodeoxyuridine (BrdU) followed by fluorescence microscopy (Fig. 1B). The "replication banding" pattern coincides almost exactly with the trypsin-Giemsa banding pattern routinely used by mammalian cytogeneticists (Fig. 1B) (*3–6*). The AT-specific fluorochromes (A, adenine; T, thymidine), such as quinacrine or Hoechst 33258, also give the same euchromatic banding pattern because the early-replicating DNA is GC-rich (G, guanine; C, cytosine) and the late-replicating DNA is AT-rich (*4*).

Very late replication is traditionally equated with genetic inertness. Constitutive heterochromatin, such as blocks of satellite DNA, and facultative heterochromatin, such as the inactive X chromosome in mammalian females, replicate late and are genetically inert (*7*). Mueller *et al.* (*8*) first showed distinct early- and late-replicating DNA fractions in euchromatin (*9*) and subsequently postulated that only the early euchromatin had active genes (*10*). Many geneticists believe that all genes are confined to the early-replicating, Giemsa-light bands, and that the later-replicating, Giemsa-dark bands, although euchromatic in the strict sense, are devoid of genes and rich in middle repetitive sequences (*11, 12*).

A number of investigations have determined the time of replication of specific genes, all of which were early (included in Table 1). Others have shown that various mutagens are most effective when introduced during the first half of S phase (*10, 13*). Stambrook and Flickinger (*14*) presented cytological evidence that the time of replication of particular DNA sequences might change during development, and suggested that ". . . RNA molecules synthesized by one cell type and not another would be coded for by genes which would replicate early in the S period in the first case, and later in the S period in the second case" (*14*, p. 101; see also *15*). In spite of these concepts, critical data concerning the functional importance of early- as compared to late-replicating euchromatin are lacking. We have described a method for fractionating early- and late-replicating DNA's from V79-8 hamster cells and characterized these DNA fractions with respect to DNA reassociation kinetics, complementarity to total polyadenylated RNA, and chromatin sensitivity to deoxyribonuclease I (*4*). Early- and late-replicating DNA were similar in these respects. In this article, we describe specifically the occurrence of protein-coding and middle repetitive (MR) DNA sequences in early- as compared to late-replicating DNA in V79-8 and HeLa cells. We present evidence that genes which are potentially active in a given cell type replicate early in that cell type, and that genes which are permanently inactive replicate late. We suggest that the portion of the mammalian genome that contains tissue-spe-

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