Assembly Associated with the Cytomatrix

ALICE B. FULTON
Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

ABSTRACT Assembly in vivo has been studied both for endogenous cytoskeletal proteins and for several classes of viruses. Autoradiography of cytoskeletal proteins has shown that many associate with the cytoskeletal framework close to the time and place of synthesis. The cytoskeletal proteins rearrange after association with the cytoskeletal framework. Rearrangement in symmetrical giant cells occurs in a centrifugal and coherent pattern. Many of the cytoskeletal proteins associate cotranslationally, as shown by their puromycin resistance in a cell-free translation system. The assembly of several groups of viruses has been shown to be associated with various components of the cytoskeleton; whether such assembly is cotranslational has not yet been addressed directly.

The conditions of assembly in the intracellular milieu cannot now, and may never, be exactly replicated in vitro; it is therefore imperative to complement in vitro studies of assembly by studies of assembly within the cell. Such studies have now been conducted both for endogenous and viral proteins by both morphological and biochemical methods.

The site of assembly and the subsequent rearrangement of cytoskeletal proteins can be revealed in monolayer cells by autoradiography. If a pulse of [^{35}S]methionine is followed by an appropriate extraction with the nonionic detergent Triton X-100 and fixation, autoradiography will display the location of the newly synthesized cytoskeletal framework proteins, operationally defined by the extraction procedure. The extraction probably removes from the cytomatrix proteins that undergo frequent exchange or that require high protein concentrations to stabilize their association with the cytomatrix. In 3T3 cells, this technique revealed that the majority of newly synthesized cytoskeletal proteins were located near the polyribosomes of the cell (1). This pattern was found both in cells with a simple, regular profile and in cells in which the polyribosomes were irregularly displayed. The concentration of cytoskeletal proteins over the polyribosomes was much greater than the density of proteins in the intact cell. With time, cytoskeletal proteins were found throughout the cell. However, this rearrangement was blocked by the protein synthesis inhibitor, emetine. This suggests that little exchange from the soluble fraction could be detected during the 3-h chase.

The site of assembly and pattern of rearrangement could not be studied further in cells that are irregular and frequently polarized. To quantitate the rearrangement, I used giant circular cells that either arise spontaneously in cultures of hemangioma cells or are induced by irradiation of cell lines, such as HeLa, that contain a large fraction of circular cells before irradiation. Irradiation does not itself perturb cell shape (2). Using such giant cells, I have confirmed my previous observations of the initial location of cytoskeletal proteins with polyribosomes and subsequent rearrangement. However, the greater size of these cells and their simple symmetrical shape allow more detailed analysis. Autoradiographic patterns were quantitated directly from samples by measuring optical density with a Quantimet 720 image analyzer (Cambridge Instruments Inc., Monsey, NY). The great majority of the newly incorporated cytoskeletal proteins are found in the cell center (Fig. 1). A chase leads to a redistribution of radioactivity; the radioactivity in the central region is reduced and a peripheral ring of radioactivity appears. These results indicate several features of cytoskeletal rearrangement: (*a*) the extraction procedure used must be largely specific or the pattern of radioactivity could not change with time; (*b*) the radioactivity redistributes as a coherent centrifugal pulse not by increasing homogenization throughout the cytoplasm (this pattern is similar to that of the slow components of axonal transport [3]); (*c*) this simple pattern of rearrangement is not obvious in the irregularly shaped cells sometimes found in these cultures nor in 3T3 cells, suggesting that there may be an additional component of cytoskeletal rearrangement that is a consequence of cellular polarization.

Autoradiography cannot resolve the time or place of assembly to within more than a few minutes or micrometers of synthesis. For greater detail, I developed in vitro translation systems that allow the examination of the earliest events. In such a translation system, I have shown that the association of cytoskeletal proteins with the cytoskeletal framework is specific, i.e., no normally soluble polypeptides are found on the cytoskeletal framework completed in vitro, and all but

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 NO. 1 PT. 2 JULY 1984 209s-211s

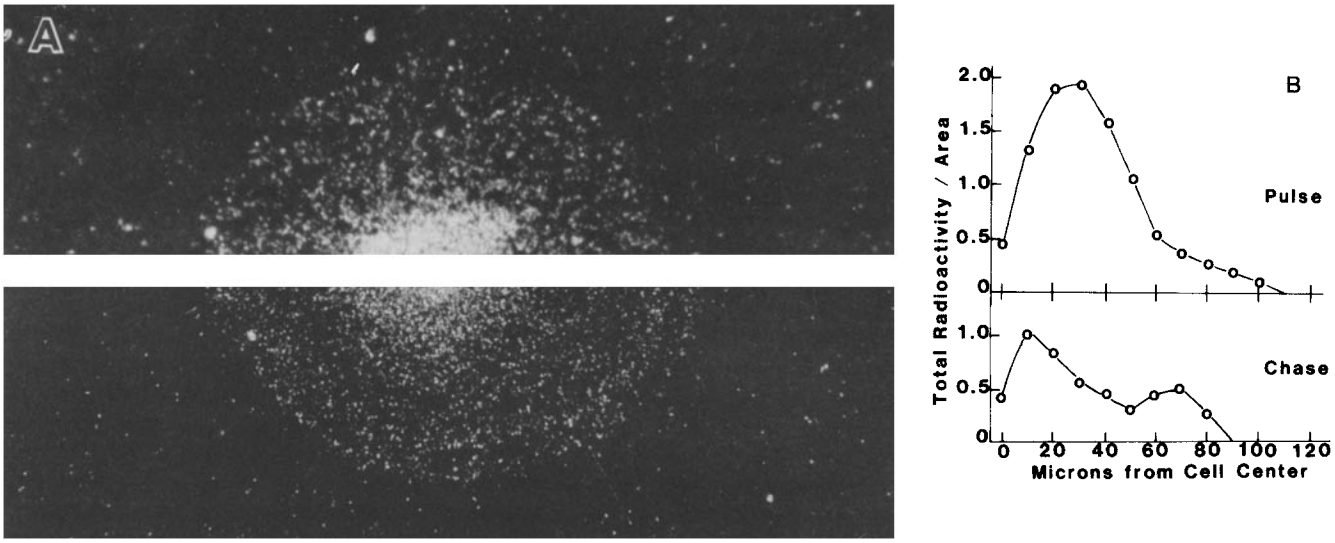

FIGURE 1 The rearrangement of cytoskeletal proteins can be measured by autoradiography. (*A*) Dark-field photomicrographs of giant hemangioma cells after autoradiography. *Top:* pulse. *Bottom:* chase. (*B*) Distribution of radioactivity in hemangioma giant cells (n = 5). Pulse: [^{35}S]methionine in the cytoskeletal framework after a 10-min pulse and a 2-min chase. Chase: treated as described for pulse, but with a 5-h chase.

two cytoskeletal polypeptides are found exclusively in the cytoskeletal fraction. Faithful assembly depends on the appropriate buffer conditions for translation. In the absence of EGTA, cytoskeletal association is reduced by more than one-half, and, in the absence of initiation in vivo, cytoskeletal association is reduced by three-fourths. Cytoskeletal association is independent of the concentration of soluble proteins at the time of translation. Finally, many components form puromycin-resistant associations with the cytoskeleton during translation (4; Fig. 2). This observation suggests strongly that many cytoskeletal proteins form cotranslational associations with the cytoskeleton. These associations depend upon the appropriate buffer conditions and are therefore specific and not a consequence of protein insolubility. More detailed studies would require the use of antibodies to the N-terminus of a protein. I am presently engaged in raising antibodies to the N-terminus of skeletal actin.

Some endogenous components have been observed morphologically to assemble by rearrangements of or interactions with the cytoplasmic matrix (5). Cardiac myofibrils appear continuous with the adjacent cytoplasmic matrix; elements of the matrix elongate and enter slender stress fibers continuous with the myofibril. In addition, numerous polyribosomes are found on the matrix, especially near the A band; these may be involved in myosin synthesis.

The other major approach to extending these studies is to exploit viruses as models of assembly. Phage assembly has served fruitfully as a paradigm for assembly from solution. It is therefore particularly significant that many animal viruses are now known to assemble extensively or solely on the cytoskeletal framework. These observations have been made in several classes of viruses, several subcellular structures, and in various cytoplasmic locations and therefore cannot be due to either a peculiarity of a single class of viruses or the adhesiveness of a particular subcellular component.

Polio virus exploits the cytoskeletal framework for most stages of its assembly (6). The viral message, like the cytoplasmic mRNA, is associated with the cytoskeletal framework, although the location of the viral message is different, i.e., peripheral rather than central. Empty virions are also found

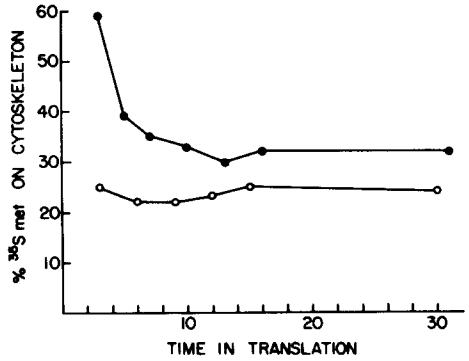

FIGURE 2 Puromycin-resistant association. During translation in vitro, many polypeptides form puromycin-resistant contacts with the cytoskeletal framework. (●) No puromycin. (○) With puromycin.

associated with the cytoskeletal framework in a different region, as is the replication complex of double-stranded RNA. However, mature virions are soluble. Thus, in this cytoplasmic virus, all stages except the mature virion are found associated with the cytoskeletal framework, although each component has a different location within it. For no stage of assembly does extraction change the intracellular location.

The iridovirus, frog virus 3, both has profound effects on the three major components of the cytoskeleton and is associated with the cytoskeleton at every stage during its assembly and release (7). At every stage of infection, viral proteins are highly localized. Early in infection, microtubules become depleted, although they still extend to the cell periphery. Intermediate filaments retract from the cell edge and surround the assembly sites for the viruses; they may play a role in the formation of assembly sites. Stress fibers disappear about the time that assembly sites form. However, late in infection microfilaments are found in bundles and projections from the cell, which terminate in virus particles and may be involved in virus release. The assembly sites were found associated with Triton-extracted cytoskeletal frameworks at the same location as in intact, unextracted cells. Thus, at all stages of virus production, the viral proteins are associated with specific cytoskeletal components, and the cytoskeletal element

involved changes over time. Virus production as a process also alters the status of the cytoskeleton.

Vaccinia virus contains a phosphorylated basic 11,000-dalton polypeptide that is exposed on mature viruses (8). This protein interacts with actin-containing cytoskeletal structures, whether or not cells are treated with drugs to block virus replication. The viral protein is detected on stress fibers in intact cells and is associated with a Triton-extracted cytoskeletal framework; it appears to be the viral protein that connects the virus to the cytoskeleton during assembly. In the absence of virus replication, the protein is still found localized both on stress fibers and in viral domains. Because of its discontinuous association with stress fibers, it may in fact associate with an actin-associated protein.

Herpesvirus assembles in the nucleus. It is therefore particularly striking that Quinlan and Knipe (9) have described a transport process for two herpes viral proteins that are associated with the cytoskeletal framework from the time of synthesis to their binding in the nucleus. The major capsid protein and the major DNA-binding protein have no detectable soluble pool. The isolated proteins have been shown to be freely soluble, and exogenously supplied viral proteins do not adhere to the cytoskeletal framework, thus excluding this association as an adventitious or artifactual binding. Although the authors consider the possibility of cotranslational association, none of their experiments directly addresses that question.

Vesicular stomatitis virus exploits the cytoskeletal framework differentially in locating its virion proteins.[1] For example, the bound N-protein associates with different cytoskeletal proteins during assembly; its rearrangement is blocked by emetine. Each virion protein displays a characteristic sequence of cytoskeletal association during virus assembly and through budding.

Clearly, viruses from many major groups exploit the cytoskeleton during assembly. Different viruses form specific associations to different components of the cytoskeleton, even some viruses that assemble in the nucleus. As yet unresolved is the possibility that some stages of these assembly processes occur cotranslationally.

These cases of viral assembly in the cytoplasmic matrix are unambiguous. Related assembly processes may be involved when the following observations are made: highly localized patterns of nonmembrane viral proteins or components; viral protein in "particulate" fractions after detergent treatment; cytoskeletal rearrangement during virus infection. These are common adjuncts to virus production; virus assembly on the matrix is probably common.

How can the observations of endogenous and viral assembly occurring on the cytoskeleton be reconciled with observations of microinjected fluorescent proteins? Microinjection can be used to observe only those cellular structures that can incorporate proteins from solution, and in principle could never be used to examine a fraction that was not in exchange with a soluble portion of the cell. In addition, such experiments cannot distinguish between a dynamic exchange process, such as treadmilling, and an irreversible assembly process that is limited either by the availability of free sites for assembly or by proximity to the time of synthesis. To distinguish between these two processes, it is necessary to measure also the exit from structures of such proteins. It is now known that in actin filaments, under physiological conditions, exchange primarily takes place at the ends of filaments. There is therefore no intrinsic contradiction between the two processes of assembly described here. The experiments discussed above deal with the initial act of assembly, whereas microinjection experiments are performed to examine the ability of a soluble, assembly competent protein to enter the skeleton at any time during its lifetime. It is notable that the adhesion plaque proteins, α-actinin and vinculin, become incorporated exceedingly slowly into structures, taking hours to become localized (10, 11), whereas the structures themselves form and disappear in 5 to 10 min (12). This is additional evidence that for some cytoskeletal proteins exchange with the soluble phase is a relatively rare event, whereas rearrangement topologically within the cell is a common one. To obtain a detailed description of the assembly of the cytoskeleton, it will be necessary to pursue both the initial association of proteins with the cytoskeleton and the subsequent events of exchange under the constraints found within the cell.

Assembly associated with the cytomatrix was not predicted by most current concepts of protein-protein interactions in the cell or the cytoskeleton. Several aspects of the cell make such assembly less surprising than it first appeared. First, the protein concentration in the cell is far higher than that found in most in vitro assembly assays. This increases the extent of assembly both by mass action and by the excluded volume effects discussed by Minton (13). Second, the complexity of the cell favors assembly; the presence in it of essentially insoluble components, such as intermediate filament proteins, that in turn associate with otherwise soluble proteins leads to a three-dimensional structure, many of whose components taken in isolation are soluble. Finally, protein folding is much faster than protein synthesis; it is physically possible that domains of proteins may fold and associate with other elements before translation is complete. All of these aspects must be kept in mind when framing and testing models for assembly in the cytomatrix.

This work was supported by grants from the American Heart Association and the Muscular Dystrophy Association.

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