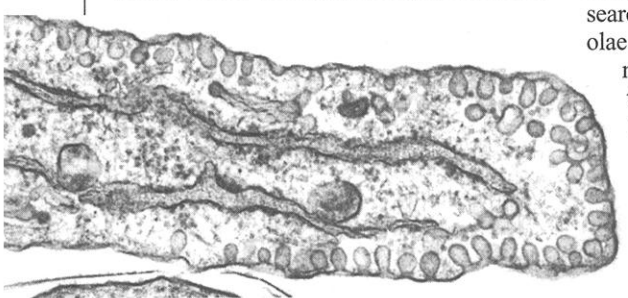
Caveolae: A Once-Elusive Structure Gets Some Respect

These membrane "domains" appear to play several important roles in the cell. But do they really perform all the functions attributed to them?

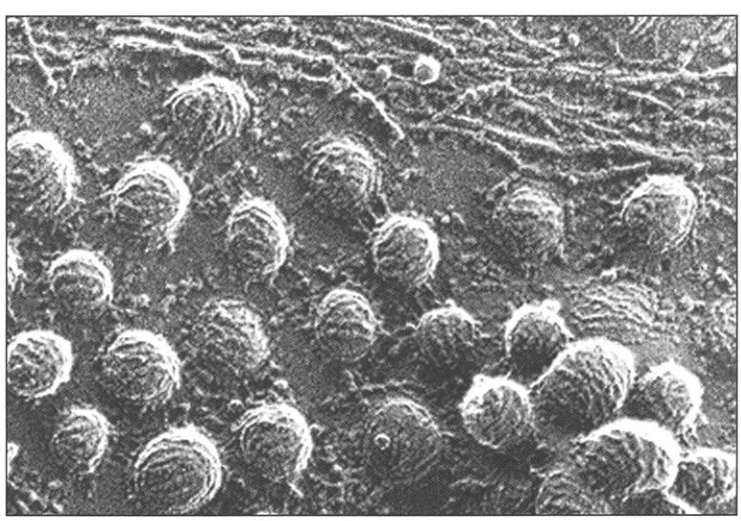
Cell biologists trying to figure out how cells regulate their many activities have traditionally focused on proteins and the genes that encode them. But studies in several labs over the past few years have revealed that proteins get key assists from an underappreciated cast of players. Lipids, including the oft-maligned cholesterol, apparently facilitate the normal operation of many proteins, particularly those located in cell membranes.



This conclusion derives from a new view of how membranes are organized. For years, researchers thought that proteins float more or less uniformly in the lipids that form cell membranes. But growing evidence paints another picture: Many proteins, including those involved in picking up hormonal and other signals from outside the cell and transmitting them to the interior, seem to be corralled in certain regions, or "domains," which have a different composition from that of the surrounding membrane. These domains, known as rafts and caveolae, are particularly rich in cholesterol and another type of fatty molecule, sphingolipids.

Rafts and caveolae have been linked to several activities, including transporting materials into and through cells, and organizing the cell's numerous signal-transduction pathways into a coherent whole. Moreover, recent findings suggest that perturbations in the functions of these cellular structures may lead to a wide range of diseases—including cancer, cardiovascular illness, and a rare form of muscular dystrophy (see sidebar on p. 1864).

But even as evidence builds that rafts and caveolae play important roles, the field is divided by a controversy over which of these membrane domains does what. Some researchers ascribe far more functions to caveolae than to rafts, whereas others—probably now in the majority—think that many of the signal-transduction functions in particular reside in rafts. As Robert Parton of the University of Queensland in Brisbane, Australia, says of caveolae, "We still don't know exactly what [they do]." Resolving the issue would give cell biologists a better understanding of how cells detect, and in particular integrate, the signals that control virtually all their activities.



Spelunker's delight? As shown in the electron micrograph (*top left*), some cells have numerous flask-shaped "little caves," or caveolae. The striated coat of caveolae, visible in the rapid-freeze, deep-etch image (*above*), may help the structures attain their shape.

The uncertainty persists despite the fact that caveolae were first recognized almost half a century ago. Electron microscopist Eichi Yamada, then at the University of Washington, Seattle, and cell biologist George Palade, then at the Rockefeller Institute in New York City and now at the University of California, San Diego, independently discovered the structures in the mid-1950s as flask-shaped indentations of the cell membrane. To Yamada, the structures resembled small caves, so that's what he called them—in Latin, caveolae.

Because Palade originally detected caveolae on endothelial cells, which line the insides of blood vessels, he proposed that caveolae might pick up materials from the blood and transport them through the cells, a process known as transcytosis. Work by his team, and by cell biologists Nicoli Simionescu and Maya Simionescu at the Institute of Cellular Biology and Pathology in Bucharest, Romania, during the 1970s and 1980s, supported that idea.

The evidence wasn't airtight, however, primarily because researchers at that time couldn't follow caveolae all the way through endothelial cells. Indeed, caveolae received little attention for decades after their discovery, mainly because researchers lacked a handle for isolating the membrane structures and following their activities.

But that situation began to change in the early 1990s when two teams identified a protein, subsequently named caveolin-1, that localizes in caveolae. (One team included Richard Anderson of the University of Texas Southwestern Medical Center in Dallas and John Glenney of the University of Kentucky School of Medicine in Lexington; the other was led by Kai Simons, who was then at the European Molecular Biology Laboratory in Heidelberg, Germany, and is now at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden.)

The discovery of caveolin-1 was critical, says Michael Lisanti of the Albert Einstein College of Medicine in New York City, because it was a marker protein for the caveolae organelle. Indeed, Simons, working with Parton, showed that caveolin-1 is necessary for formation of the typical flask-shaped caveolae seen in electron micrographs. Since the protein's identification in the mid-1990s, "the field has really exploded," Parton says.

Common carrier?

In one prominent line of investigation, researchers are trying to pin down the role of caveolae in transporting materials into and through cells, an endeavor that should be

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aided by the recent creation of two strains of mice in which the gene for caveolin-1 has been inactivated, or "knocked out." The work has been described in the past few weeks by two teams, one led by Teymuras Kurzchalia of the Max Planck Institute of Molecular Cell Biology and Genetics, and the other by Lisanti.

The cells of the knockout animals show no signs of typical flask-shaped caveolae. Even so, Lisanti says that although his team's animals have lost the ability to move some proteins through endothelial cells, they can still move others—evidence that caveolin-1 is needed, at least sometimes, for transcytosis. Jan Schnitzer of the Sidney Kimmel Cancer Center in San Diego suggests, therefore, that caveolae might be useful medically for moving drugs, particularly proteins, across tissue barriers, and his team is now exploring that possibility. (The Kurzchalia team's results appeared in the 28 September issue of *Science*, and those of the Lisanti group are in the 12 October issue of the *Journal of Biological Chemistry*.)

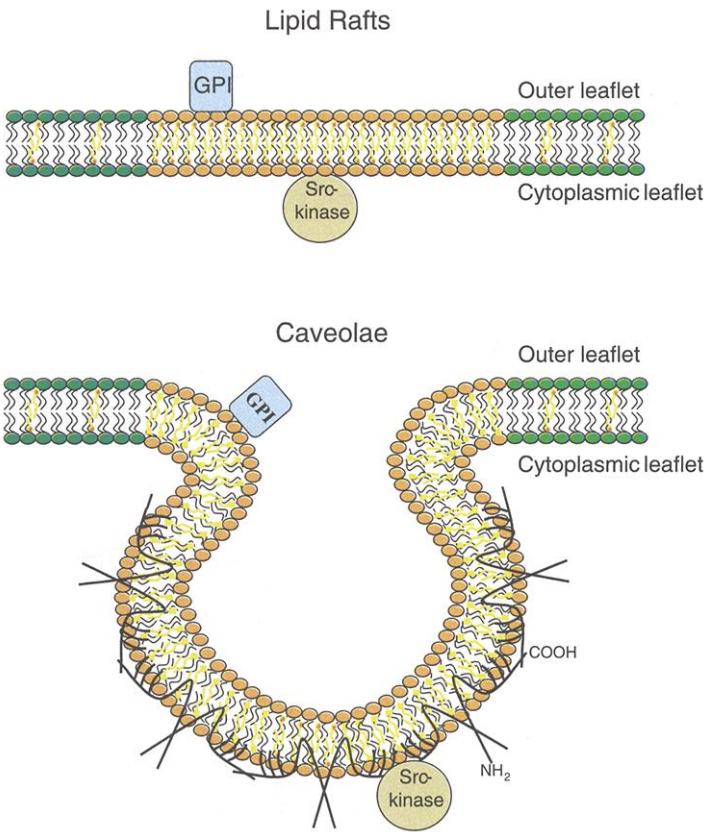
Evidence from another line of research has also strengthened the case for the role of caveolae in transcytosis. In as yet unpublished work, Schnitzer and his colleagues produced an antibody that specifically recognizes caveolae and then attached it to tiny gold particles, which are electron-dense and thus show up on electron micrographs. When the researchers washed this tracer antibody into rat lungs, or injected it into the animals' veins, they could see that it was picked up by the caveolae of endothelial cells and transported through them.

Caveolae may be involved in other forms of transport as well. In work begun before they found caveolin-1, the Anderson team was looking at the receptor that brings the B vitamin folic acid into cells and unexpectedly found that the folate receptor is located in caveolae and is carried into the cell by those structures. "As often happens in science, we started out thinking we were doing one thing and ended up doing something else," Anderson says.

Another area that has been receiving attention lately is the possible role of caveolae in cholesterol transport within the cell. If such involvement does occur, disruptions in the system might contribute to atherosclerosis and heart disease.

Platforms for efficiency

The most active research area now focuses on the possibility that caveolae are key players in signal transduction. Researchers in several labs have found that the structures contain numerous proteins involved in picking up signals from hormones, growth factors, and other molecules. These include the receptors for insulin and epidermal and platelet-derived growth factors (PDGF), for example, as well as for associated proteins—the so-called G proteins and kinase enzymes, among others—that detect when receptors have been activated and then alert other molecules in the cell interior accordingly.

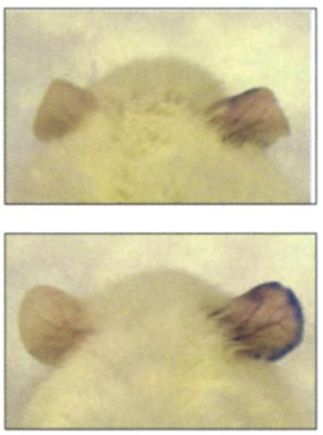


Membrane domains. Both rafts (top) and caveolae (bottom) may be rich in cholesterol and sphingolipids (yellow); many—but not all—researchers think that caveolae form from rafts by addition of interacting molecules of the protein caveolin-1 (black).

These discoveries led some researchers, including Lisanti and Anderson, to propose that caveolae serve as platforms for integrating the cell's signal-transduction machinery. They reason that it's inefficient for randomly distributed proteins to somehow piece together an ad hoc pathway in response to each incoming signal; instead, pathways are likely to be preassembled. This would also facilitate the "cross talk" that often occurs between different pathways, as the components for such interaction could be located in the same caveolae. But there is a "critical question," Schnitzer says. "Is the interaction physiologically significant? Molecules can interact in the test tube but not [necessarily] in the cell."

In some cases, particularly one involving an enzyme called endothelial nitric oxide synthase (eNOS), there is good agreement that the significance test can be met. As its name implies, eNOS makes nitric oxide in endothelial cells in response to a variety of stimuli, including hormones or neurotransmitters (such as bradykinin and acetylcholine) and the stress exerted on blood vessels by high blood pressure. Nitric oxide helps counteract that stress by relaxing the blood vessels, allowing easier blood flow; it has other effects as well, such as fostering new blood-vessel formation and wound-healing. Several researchers, including Anderson, Lisanti, and Schnitzer, and also Thomas Michel of Brigham and Women's Hospital in Boston and William Sessa of Yale University School of Medicine, have shown that eNOS is bound to caveolin-1 in endothelial-cell caveolae and that this binding keeps eNOS inactive.

For example, in work reported in the December 2000 issue of *Nature Medicine*, Sessa and his colleagues used a fruit fly protein to carry the segment of caveolin-1 that binds eNOS into rings of mouse aorta maintained in culture. When the researchers then added acetylcholine to the cultures, they found that the aortic rings



Anti-inflammatory. The right ear of the control mouse (*bottom*) shows blood-vessel dilation and leakage induced by an inflammatory material. But injection of a peptide that mimics caveolin-1—by tying up the enzyme eNOS and thus preventing nitric oxide production—reduces those inflammatory changes.

CREDITS (TOP TO BOTTOM): F. GALBIATI, B. RAZANI, M. P. LISANTI, *CELL* 106, 403 (2001); M. BUCCI *ET AL.*, *NATURE MEDICINE* 6, 1362 (2000)

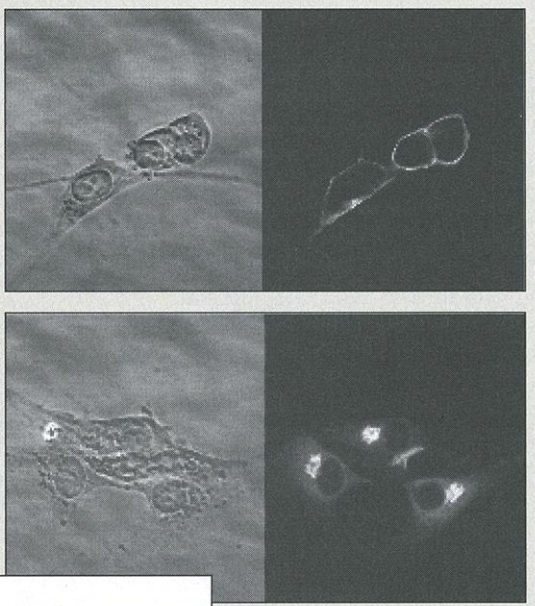
Caveolin-3 Helps Build Muscles

The cell-membrane structures called caveolae and their associated proteins—the caveolins—play a role not only in transporting materials and signals into cells (see main text) but in building the body's muscle cells. Indeed, defects in muscle caveolin have been linked to a rare form of muscular dystrophy.

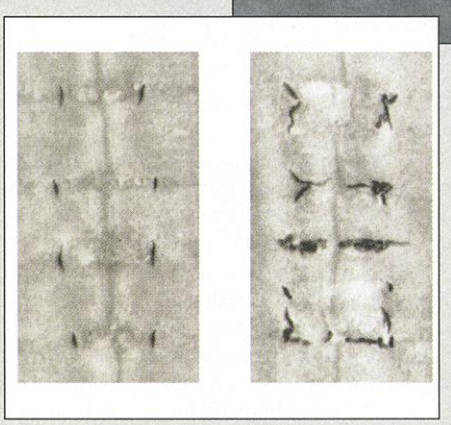
Muscle caveolin, also known as caveolin-3, was originally identified in the mid-1990s by two teams, one led by Michael Lisanti at the Albert Einstein College of Medicine in New York City and the other by Robert Parton at the University of Queensland in Brisbane, Australia. The Albert Einstein group had been searching the gene databases for relatives of the one caveolin gene known at the time, Lisanti recalls. The researchers came up with two, and when they looked to see where those genes are active, the one making caveolin-3 turned out to be highly expressed in all three types of muscle—skeletal, cardiac, and smooth—but not in other cell types.

The researchers also began picking up hints that caveolin-3 might be involved in muscle formation. For example, Parton and his colleagues found in 1997 that during development the protein associates temporarily with one of the structural features of muscle cells, the so-called transverse tubules, which form at the ends of the repeating units that make up the cell's contractile machinery. Meanwhile, Lisanti's group found evidence that caveolin-3 associates with dystrophin, a protein mutated in Duchenne muscular dystrophy.

Such findings raised the possibility that caveolin-3 mutations might also contribute to muscular dystrophy; and in 1998 Lisanti teamed up with Carlo Minetti's group at the University of Genoa, Italy, to investigate. The researchers found mutations in the protein's gene in affected members of two families with histories of limb-girdle muscular dystrophy, a mild form of the disease characterized by overdevelopment of the calf muscles and moderate weakness of the muscles in and near the body trunk. At about the same time, Louis Kunkel's group at Children's Hospital in Boston linked changes in the gene to muscular dystrophy cases with similar symptoms.



Misdirected. As shown by the bright stain in the upper right micrograph, normal caveolin-3 is located in the cell membrane. But a mutant form linked to muscular dystrophy is trapped inside the cell.



Muscle builder. Knocking out the gene for caveolin-3 (right micrograph) causes the transverse tubules (black stain) of skeletal muscle to develop abnormally. Normal muscle is at left.

Although some of the apparent mutations found in caveolin-3 have turned out to be harmless genetic variations, others cause the protein to misfold, leading to its destruction by the cell, Lisanti says. Additional evidence that such loss can cause limb-girdle muscular dystrophy came when his group and that of Yasuko Hagiwara at the National Center of Neurology and Psychiatry in Kodaira, Japan, inactivated the gene in mice. The researchers found that the animals experience muscle degeneration similar to that of human patients. "Now we can conclusively say that loss of caveolin-3 causes the muscular dystrophy," Lisanti says.

Too much caveolin-3, however, can disrupt muscle structure as well. Lisanti and co-workers found that when they genetically engineered mice to overproduce the protein, those animals developed pathological changes much like those of Duchenne muscular dystrophy. This may occur because excess caveolin-3 interferes with dystrophin's ability to reach its normal location in the cell membrane. **–J.M.**

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produced much less nitric oxide than control tissue did and also relaxed less. Activation of eNOS had been blocked. In addition, when injected into mice the caveolin-1 peptide blocked inflammation induced in the animals' ears by mustard oil, a result that indicates that it could have medical benefits.

Researchers have found that signals activate eNOS by first causing its dissociation from caveolin-1. This takes several steps, the first being an increase in the number of intracellular calcium ions. These bind to and activate a protein called calmodulin, which in turn binds to eNOS, causing it to release its grip on caveolin-1 and become active. "The other thing that's interesting," Michel says, "is that many of the receptors that activate eNOS are also in caveolae." This suggests the presence of preassembled pathways and the potential for cross talk.

Tumor suppressor

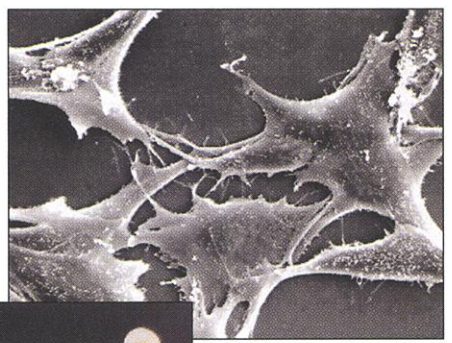
Caveolae and caveolin-1 also appear to be involved in signaling pathways that control cell growth—a function that suggests a link to cancer. "Almost all normal cells have caveolin-1," Lisanti says, "but when they are transformed by active [cancer-causing] oncogenes, caveolin-1 expression is down-regulated." The gene's expression is also low in cells derived from human cancers, including breast cancer. When researchers, including Schnitzer and Lisanti, put an active caveolin-1 gene back into the cancer cells, however, it suppresses their abnormal growth and other cancerous behavior.

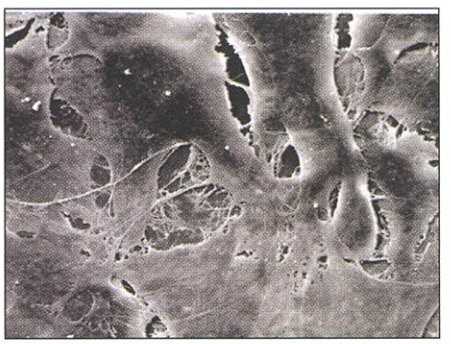
Lisanti suggests that caveolin-1 works in this situation, much as it does with eNOS, by binding to and inhibiting proteins that drive cell division. The Anderson team, among others, has shown that all the proteins needed for cells to respond to PDGF—including the PDGF receptor and certain kinase enzymes that transmit growth signals to the cell interior—are located in caveolae. Lisanti adds that caveolin-1 binds to kinases in their catalytically active sites, thereby blocking their activity.

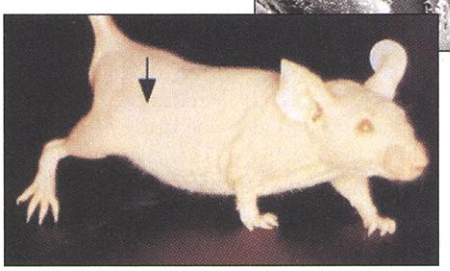
The Lisanti team also found evidence that caveolin-1 is a suppressor of human tumors. Two years ago, the researchers

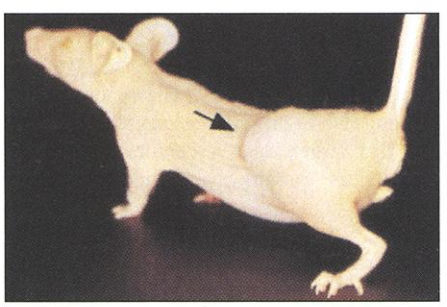
mapped the caveolin-1 gene and the gene for caveolin-2, a closely related protein also found in caveolae, to a region of the genome that is often deleted in human cancers—a typical sign that the region contains a tumor suppressor.

But perhaps most intriguing, a team led by Kazuhiko Hayashi of the Nagoya School of Medicine in Japan reported in the 15 March issue of *Cancer Research* that the caveolin-1 gene was mutated in 16% of the 92 human breast cancers they examined. What's more, the mutation hit the particular segment of the protein that binds to other proteins—a location where it could interfere with its binding ability, thus allowing kinases or other proteins involved in growth to be active when they shouldn't.









Tumor suppressor. In contrast to the normal cells (*left*), cells in which caveolin-1 is inhibited (*right*) pile up on one another in culture. This increased growth is reflected in their ability to produce large tumors in mice (bottom animal). The cells in which caveolin-1 is active (top animal) do not form a tumor.

A domain of its own?

Work on the knockout mice has supported the idea that increased cell proliferation may be expected when caveolin-1 is absent. In living animals, this was manifested primarily by extra layers of cells in the lungs. Lisanti and his colleagues also found that embryonic fibroblasts from the knockout mice proliferated twice as fast as those from controls. Exactly why this happened is currently unclear, however, as it did not seem to involve the kinase activation previously observed in other cultured cells.

The eNOS work also received support from the caveolin-1 knockouts, as the animals clearly suffered from the types of defects that would be produced by the enzyme if it were overactive. For example, aortic rings from the animals showed a much greater relaxation response to acetylcholine than rings from unaltered animals. "The one phenotype I would have predicted is exactly the one they have," says Michel.

Although the knockouts support some of the ideas about the function of caveolae, several researchers have expressed surprise that the animals survived at all, given the large number of functions, particularly in the area of signal transduction, attributed to the structures. The knockout "was a real surprise to the field. We imagined it would be lethal to the embryo," Parton says. He and others suggest that many of the processes thought to be going on in caveolae are going on elsewhere, such as in lipid rafts.

Schnitzer says, for example, that his team found some signaling proteins in caveolae and others in rafts. And Parton adds that others may be in neither. In fact, he maintains that "for most of the signaling proteins originally claimed to be in caveolae, there is now conflicting evidence."

Indeed, there is a major controversy in the field over both the nature of caveolae and their functions. Rafts were discovered much more recently, largely as a result of work by the Simons team in the late 1980s and 1990s. Like caveolae, they are thought to be rich in cholesterol and sphingolipids, but are flat and do not contain caveolin. Most researchers in the field now think that caveolae are a subset of rafts, formed by acquisition of caveolin-1. That idea also got a boost from the Kurzchalia and Lisanti caveolin-1 knockouts, which totally lack flask-shaped caveolae.

But there are some dissenters from this view, particularly Anderson. For one, he maintains that caveolae can have any shape. To support this, he cites experiments in which his group blocked cell uptake of folate and found that the flask-shaped caveolae were no longer visible. Yet, the researchers were still able to recover them from membranes. "The fact that flask-shaped caveolae disappear doesn't mean they aren't there," Anderson says.

He even argues that caveolae don't have to contain caveolin, suggesting that some other protein in the knockouts could be pinch-hitting. "To me," he says, "[the knockouts] are strong evidence that caveolin isn't the only player."

There's currently little evidence to support the idea that caveolae can exist without caveolin-1, however, and most researchers remain skeptical. Simons, for one, says he wants to see both a flask shape and the presence of caveolin-1 before he will accept a structure as a "little cave."

What it will take to resolve the controversy is unclear, given how hard it is to isolate membrane structures. But until cell biologists do, they won't have a clear view of how signals get into the cell. In that spirit, Anderson did say one thing with which everyone would agree: "We still have a lot of work to do."

—JEAN MARX

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