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Organelle recitals

To isolate and study organelles from cells, scientists use methods that range from primitive to modern. By Alan Dove

Over 50 years ago, Albert Claude and George Palade of Rockefeller University found a way to separate the individual organelles of cells for analysis. Their method, differential centrifugation, relied on breaking the cells’ outer membranes and then sedimenting the lysate through fluids of different viscosities. This work helped start the era of modern cell biology.

The field has since blossomed and expanded in ways its founders never anticipated, but as a new generation of researchers brings modern proteomic and genomic tools to bear on organelle biology, they often find that the first few steps in their protocols have hardly changed. Dounce homogenizers, centrifuges, and sucrose gradients still feature prominently in cellular laboratories everywhere. “I don’t think we have seen any revolution in the field since Claude and Palade’s differential centrifugation,” says Luca Scorrano, scientific director of the Venetian Institute of Molecular Medicine in Venice, Italy.

Old school

Cell biology’s reliance on classical techniques can be both reassuring and annoying. The standard approaches use equipment and reagents most researchers probably already have in their labs, but figuring out exactly how to purify a particular organelle can be surprisingly difficult. Scorrano explains that newcomers to the field quickly find that methods sections in modern papers often reference work from decades earlier. “It takes quite a while to dig into the original literature to find how they really isolated [organelles],” he says.

Even with a published protocol in hand, the process may not work as expected. “There are a number of tricks which are usually passed from researcher to researcher by word of mouth,” says Scorrano. Several years ago, he and his colleagues crystallized one such artisanal protocol for mitochondrial isolation by publishing it in a peer-reviewed journal. Unfortunately, Scorrano says that few others have followed suit, because so much of the field’s technical knowledge continues to reside in oral traditions. “If you have [access to] somebody who is knowledgeable about [organelle] preparation, use them,” he adds.

When researching an organelle isolation method, investigators should also consider what they intend to do with the final product. For example, studying the physiological state of mitochondria in a rat liver at a particular time may call for a rapid but crude extraction of the organelles before they begin to degrade. Proteomic analysis of the same mitochondria would require more careful purification, with less concern about maintaining the organelles’ physiology.

Scorrano points out that the mitochondrial field also has a historical division between researchers working on protein import, who often study yeast, and those studying bioenergetics using rat liver cells. Yeast mitochondrial isolation generally involves hyperosmotic buffers that could derail the physiology of rat mitochondria.

Despite the challenges of starting from scratch, Scorrano takes a dim view of prepackaged organelle purification kits, preferring that those in his lab learn the entire process. “You might need some time to get to a level in which you can easily isolate organelles with decent purity and with decent function, but [kits] are banned in my lab,” he says.

Kitting up

Researchers with less experience in organelle isolation tend to be more open to off-the-shelf solutions, and several companies cater to that market. “These are definitely well-established protocols; there’s nothing new under the sun,” says George Yeh, product manager for protein biology at Sigma-Aldrich in St. Louis, Missouri. However, Yeh adds that “one concern is uniformity of technique from lab to lab.” Using a prepared reagent kit and protocol from an established company can improve reliability. “I think what we bring to it is consistency—that you know that every lot of the kit is going to look the same—and the ease of use,” says Yeh.

Sigma-Aldrich currently offers over a dozen different organelle isolation kits, each optimized for a specific organelle or type of analysis. “We have kits for isolation of peroxisomes or Golgi or endoplasmic reticulum, [or] chloroplasts for those working on plants,” says Yeh. For organelles that contain their own DNA, such as mitochondria and chloroplasts, researchers can follow the organelle purification with a DNA-isolation step to track subcellular genetics.

Scientists studying organelle-associated proteins increasingly use mass spectrometry after purifying the target organelle, which has added a new...
For organelles that contain their own DNA, such as mitochondria and chloroplasts, researchers can follow the organelle purification with a DNA-isolation step to track subcellular genetics.

challenge: Reagents used in some older protocols may interfere with mass spectrometers. In response, Sigma-Aldrich has launched a series of reagents and kits designed to be mass spectrometry-compatible.

Besides kits, the chemical supply giant also sells individual reagents, and Yeh concedes that ready-made kits may not always be the most cost-effective choice. “If you’re going to do it a hundred times over, I would bet you’re better off making your own kit,” he says, adding that “the market we serve [with kits] are people who do it once or twice.”

Regardless of which approach they choose, Yeh echoes Scorrano’s assessment that any lab should be able to execute competent organelle isolations once they’ve taken the time to learn and practice the procedures. “There’s always that first hurdle to jump over, but once they do that they’re on their way,” says Yeh.

Smart shopping

Over the years, some researchers and lab suppliers have also developed their own special tricks to improve organelle isolation procedures. Although most of the modifications are minor, they add up for certain types of analyses.

“Traditional methods will provide purified proteins to the extent of doing Western blots, where even a degraded protein might work . . . but when you go for any activity analysis, you will need the protein to be in its functional state,” says Payal Khandelwal, product manager for assay kits at Biovision in Milpitas, California. “That is where the more enhanced techniques come into the picture,” adds Khandelwal.

Besides protein degradation, older techniques may be less effective at separating different organelles from cytoplasmic components. That may not matter for physiological experiments, but in highly sensitive genomic or proteomic studies, the contaminants could mask the phenomena the researcher is trying to track.

Biovision is one of several companies working to solve those problems with carefully optimized organelle purification kits. “It’s not involving any extra machinery or instrumentation; it just involves some very specific and very advanced reagents which are provided along with the kit,” says Khandelwal. Other than the kit, researchers need only ordinary centrifuges and related equipment they probably have on hand.

Perhaps the most challenging part of using such kits is deciding which one will work best. Comparing the protocols to see which is the most straightforward is one approach, but companies don’t disclose the proprietary ingredients in their buffers, so it can be hard to predict which will work best for a particular lab’s needs. Khandelwal suggests asking about the expected yield of a kit and the assays required to determine whether the isolation worked. “If the client is looking at, say, mitochondrial DNA, of course DNA is going to be isolated, but how do you know whether it is pure mitochondrial DNA, or [if there is] contamination from nuclear DNA?” asks Khandelwal.

Another advantage of using kits optimized for a particular type of experiment is support. If the experiment doesn’t work, researchers who’ve used kits can call the manufacturer for troubleshooting help, whereas those who’ve mixed their own reagents will have to figure out the problem on their own.

More than the sum of its parts

Although biochemists may be anxious to separate organelles from their cellular contexts, it often pays to take a close look at the intact system first. That’s particularly true for mitochondria, which can adopt different configurations depending on the cell’s physiological state.

“We once described the mitochondrion as a small football-shaped organelle,” says James Murray, general manager of Abcam in Cambridge, United Kingdom. He adds, “We’ve come to realize that that’s actually not the case; they’re not discrete organelles necessarily.”

Indeed, depending on the cell type and its current state, mitochondria can be individual ellipsoids or may form reticulated networks that connect to other structures such as the endoplasmic reticulum and plasma membrane. “It’s a very dynamic and fluid situation,” says Murray.

That raises problems for researchers who want to study isolated mitochondria. Pulling a spaghetti-like network of membranes out of a cell is considerably harder than separating discrete organelles. Murray suggests that researchers begin by using techniques such as immunofluorescence to visualize a cell’s organization before deciding whether to break it open. If the question is whether a particular protein localizes to a particular organelle, microscopy could provide all the necessary data without requiring researchers to resort to any isolation techniques.

For those who do need to fractionate cells, Murray reiterates other experts’ advice to pick the right protocol for the ultimate analysis. Experiments that require intact, physiologically active mitochondria are among the most finicky organelle isolations. Starting with healthy, fresh cells, experimenters need to complete the entire protocol...
and analysis as quickly as possible. Efforts to revive mitochondria after storage in a freezer generally fail. Getting active mitochondria also requires one step that kit makers haven’t been able to simplify: sucrose gradient centrifugation. Researchers must mix two different concentrations of sucrose together while slowly transferring the mixture into a centrifuge tube, generating a smoothly increasing density gradient from the bottom to the top of the tube. The gradient has to be mixed immediately before use, and getting consistent results takes practice. Abcam and other companies are working on antibody-based organelle purifications that would omit the tedium of sucrose gradients, but those products are still in development. “I think maybe we’re on the cusp of taking a step forward and having affinity-based methods to isolate organelles,” says Murray.

The methyl lab
For investigators who just want to isolate mitochondrial or chloroplast DNA and don’t care about the organelles’ physiological states, that future has already arrived. Scientists at New England Biolabs (NEB) in Ipswich, Massachusetts have developed an antibody-based protocol for separating organelle and nuclear DNA. The technique exploits differences in methylation between the two pools of genetic material.

“In nature, organelar DNA is not methylated, or is methylated at a very low level,” explains Erbay Yigit, applications and product development scientist at NEB. Yigit and his colleagues created a methyl-binding protein fused to an antibody constant region, which binds DNA only if it’s relatively well-methylated. By allowing the engineered protein to bind total isolated cellular DNA, the team can precipitate the nuclear DNA from the mitochondrial or chloroplast DNA.

The researchers originally developed the protocol to separate microbial from human genomic DNA for microbiomics research, but quickly found that it works well for organelle DNA separation as well. NEB now sells the reagents in kit form, including magnetic beads covered in Protein A, which bind the antibody constant region to precipitate the methylated nuclear DNA without a centrifugation step. “This protocol is really straightforward, so you can start with extracted DNA [and] there’s nothing to be scared of,” says Yigit. After a standard DNA purification protocol, researchers need to perform only a few additional steps to separate the organelle and nuclear DNA fractions.

Plant biologists may still have to deal with a tricky separation problem, however. Yigit explains that the NEB kit leaves mitochondrial and chloroplast DNA in the same fraction. “DNA from these organelles is very similar,” he says.

Download the app
Although the growing collection of kits certainly speeds many types of experiments, some scientists may be able to get a head start on their subcellular studies without even getting their hands dirty. “If you know the protein sequence, you can deduce, in some cases very accurately, where that protein is located,” says Fiona Brinkman, professor in the School of Computing Science at Simon Fraser University in Burnaby, British Columbia, Canada.

Brinkman and her colleagues have developed a software application called “PSORT” to make such predictions. The team initially focused on bacterial and archaeal proteins, but a branch of the program also works for eukaryotic cells. “We’re [increasingly] appreciating [that bacteria] have organelle-like structures,” says Brinkman.

PSORT uses a machine learning algorithm, which takes examples of well-proven protein localizations and then extrapolates to predict which other proteins will localize similarly in cells. Brinkman explains that “there’s a lot of power in getting more lab data” to train the algorithm further, but the search for those data has revealed some of the pitfalls of fractionating cells. Brinkman emphasizes that researchers need to analyze all of the fractions they isolate, rather than just the one that interests them most. “You can have contamination from other fractions, so you want to see that [a protein] is actually located predominantly in one fraction,” she says.

After being fed carefully vetted data, however, PSORT has matured into a powerful tool. “It’s impressively accurate now for the organisms that have been traditionally well-studied,” says Brinkman, adding that her team is now extending the program to make predictions for a broader range of organisms. Since its development, PSORT has been downloaded thousands of times and cited in numerous publications.

PSORT’s popularity suggests that many investigators with little bioinformatics training are using it. Brinkman encourages that, but cautions users to read the associated publications. “For any computational method . . . make sure you’re aware of the accuracy and how that’s been investigated for a particular method,” she says.

That advice echoes what Scorrano says about traditional differential centrifugation: “It’s easy. [But] it’s much more complicated to interpret [the results].”

Alan Dove is a science writer and editor based in Massachusetts.

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