Researchers reveal the complex assembly process involved in DNA virus replication

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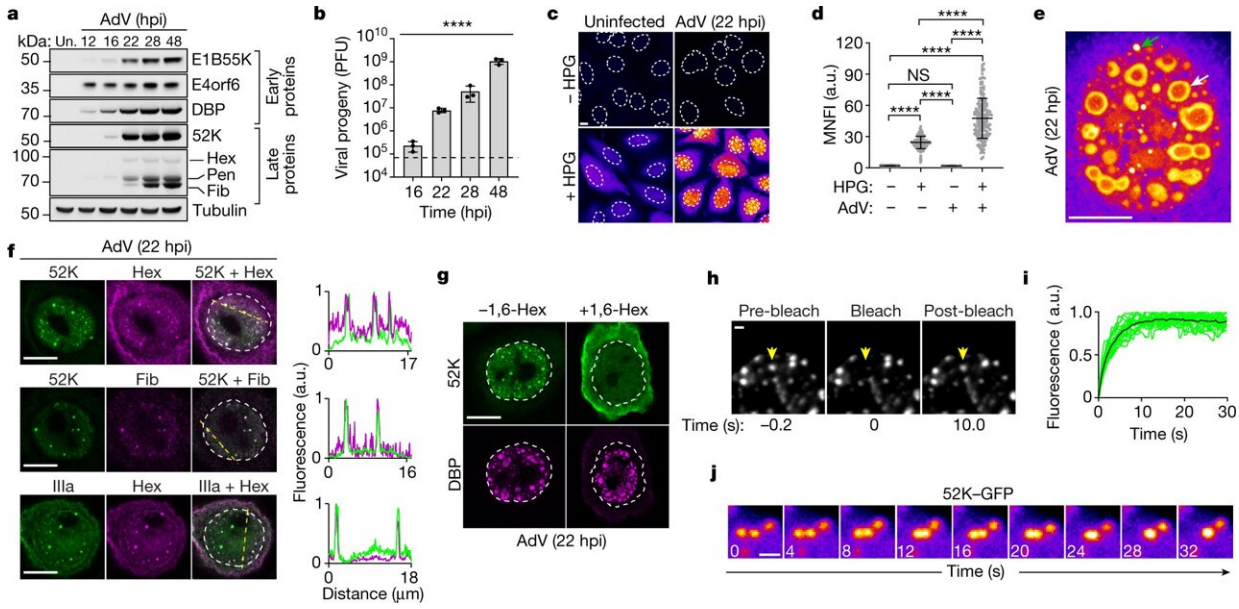

The viral 52K and capsid proteins form NBs with characteristics of dynamic BMCs. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-05887-y

"Which came first, the chicken or the egg?" Scientists have long faced a similar question about how human adenovirus replicates: "Which comes first, assembly of the viral particle, or packaging of the viral genome?"

Now, in a new study published today in *Nature*, researchers at Children's Hospital of Philadelphia (CHOP) have answered that question, showing that viral proteins use a process called phase separation to coordinate

production of viral progeny.

"This study answers a fundamentally important question: how a viral nucleic acid gets inside a particle so that viral offspring can be delivered to cells," said Matthew Charman, Ph.D., a research associate in the Weitzman Lab at Children's Hospital of Philadelphia. "These findings have broad implications, from potential therapeutic interventions to improved gene therapy delivery, in addition to expanding our understanding of basic cell biology."

Viruses hijack host cellular processes to replicate and produce infectious offspring that are key for viral spread and transmission. To do so, they must both replicate their viral genomes and package those genomes into viral particles, so that the infectious cycle can continue. However, little is known about how genome replication, particle assembly, and genome packaging are coordinated in the crowded nuclear environment.

"If we think of viral replication as an old-fashioned milk assembly line, we know how the milk bottles are formed and that they come out filled, but prior to this study, the process of filling them was somewhat of a black box," said senior author Matthew D. Weitzman, Ph.D., a professor in CHOP's Department of Pathology and Laboratory Medicine.

"Our findings suggest that the viral particle forms around the viral genome. Extending the analogy, many have assumed that the bottle must be made before being filled, but it turns out the bottle is actually formed around the milk. Led by Dr. Charman, we have shown that a biophysical process known as phase separation allows this process to occur in an orderly, coordinated fashion."

Emerging evidence suggests that membraneless compartments form inside virus-infected cells by phase separation. These membraneless compartments, known as biomolecular condensates (BMCs), can

regulate biological processes by concentrating or sequestering biomolecules in an enriched dense phase, while limiting their concentration in the light phase.

Although BMCs have been linked to several viral processes, there was insufficient evidence that phase separation contributes functionally to the assembly of infectious viral offspring in infected cells.

To investigate the potential role of BMCs in this process, the researchers studied adenovirus, a nuclear-replicating DNA virus. Because the adenovirus proteins involved in genome replication are distinct from those involved in particle assembly and genome packaging, the researchers reasoned focusing on this virus would allow them to dissect and more easily identify the role of phase separation in specific viral processes.

Through a variety of techniques, including homopropargylglycine (HPG) labeling and fluorophore click chemistry, the researchers demonstrated that the adenovirus 52 kDa protein—a dedicated assembly/packaging protein—makes its own membraneless structures through phase separation and plays a critical role in the coordinated assembly of new infectious particles.

They showed that not only does the 52 kDa protein organize viral capsid proteins into nuclear BMCs, but also that this organization is essential for the assembly of complete, packaged particles containing viral genomes.

Additionally, the researchers performed experiments with a mutant adenovirus lacking the 52 kDa protein and showed that incomplete capsids formed in the absence of viral BMCs. Thus, the researchers were able to show that by altering the formation of these membraneless structures within the cell, the "assembly line" producing viral offspring

no longer functioned properly.

"Now knowing these steps, the question becomes: could we reengineer viruses based on this biological process to, for example, become better delivery vehicles for innovations like gene therapy?" Dr. Charman said. "Understanding how viruses are made opens up a world where we could not only potentially target those viruses more effectively in the future but also create gene therapy tools that lack the limitations of current delivery approaches."

More information: Matthew Weitzman, A viral biomolecular condensate coordinates assembly of progeny particles, *Nature* (2023). DOI: 10.1038/s41586-023-05887-y. www.nature.com/articles/s41586-023-05887-y

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