Biologists take snapshot of fleeting protein process

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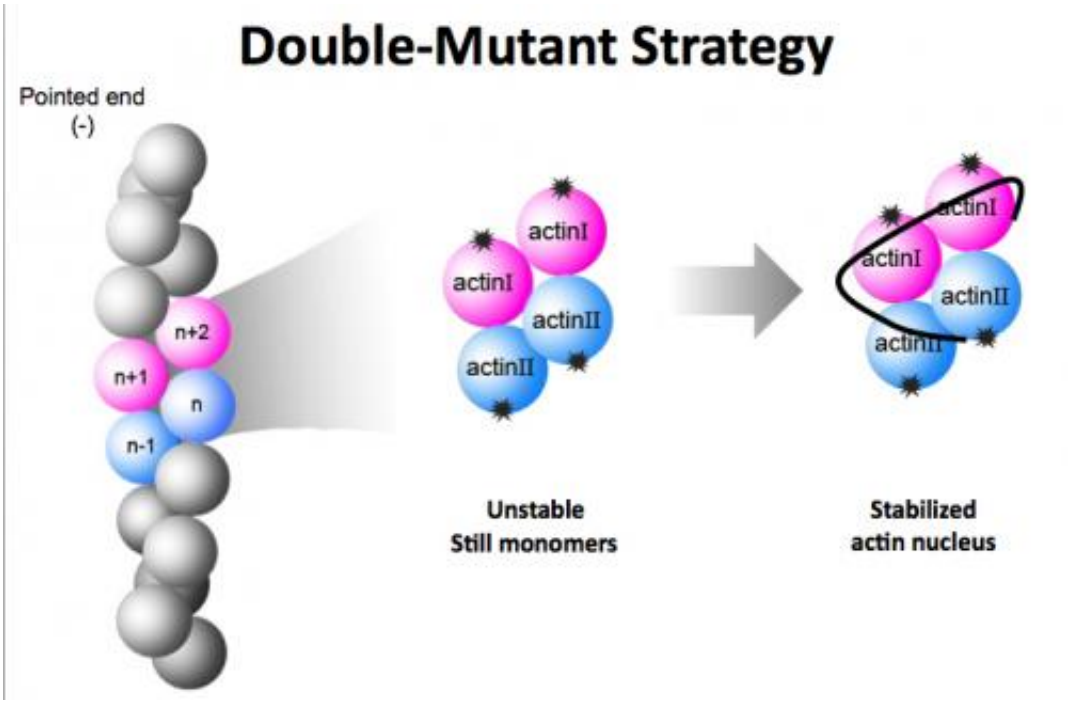


To decipher the structure of the F-actin nucleus, researchers used a dual-mutant strategy. They created two mutant versions of actin monomers that could bind together to form a nucleus but could not bind with additional monomers to form the F-actin polymer chain. Credit: J. Ma/Rice University

Structural biologists from Rice University and Baylor College of Medicine (BCM) have captured the first three-dimensional crystalline snapshot of a critical but fleeting process that takes place thousands of times per second in each human cell. The research appears online today

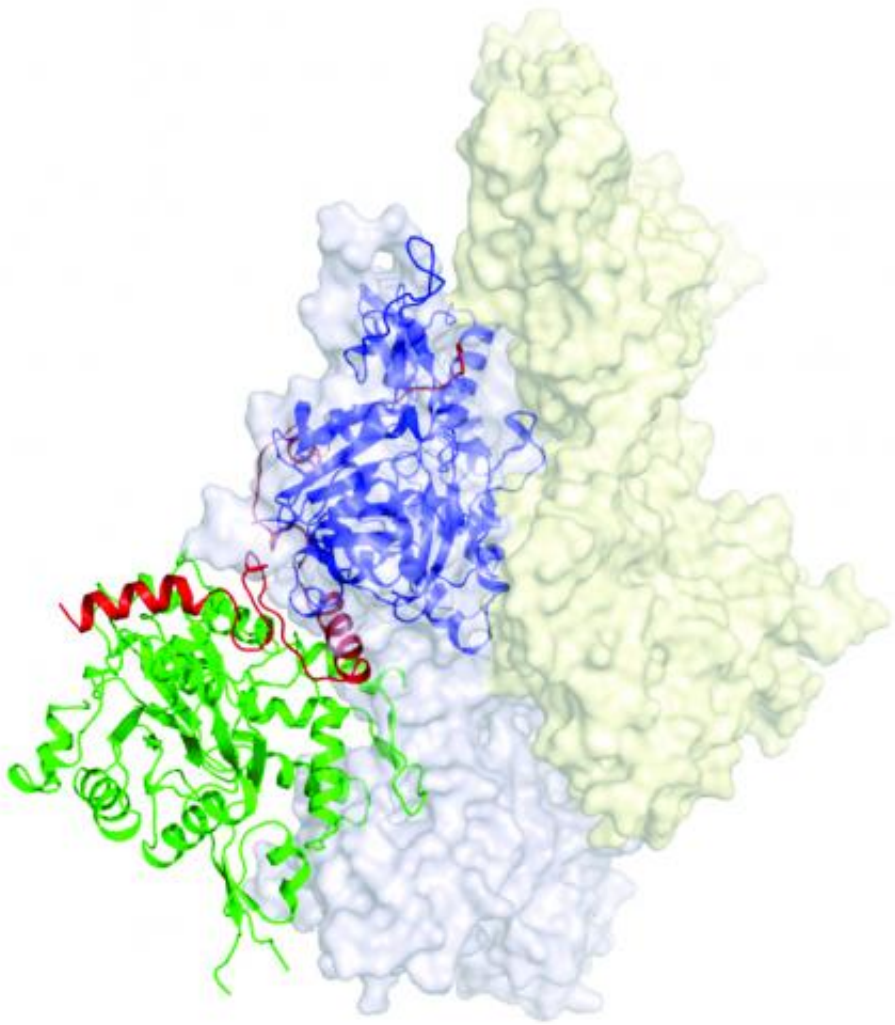
in the journal *Cell Reports* and could prove useful in the study of cancer and other diseases.

The biological "freeze-frame" shows the initial step in the formation of actin, a sturdy strand-like filament that is vital for humans. Actin filaments help cells maintain their shape. The filaments, which are called F-actin, also play key roles in muscle contraction, cell division and other critical processes.

"One of the major distinctions between cancerous cells and healthy cells is their shape," said study co-author Jianpeng Ma, professor of bioengineering at Rice and the Lodwick T. Bolin Professor of Biochemistry at BCM. "There is a correlation between healthy shape and well-regulated cell growth, and cancer cells are often ugly and ill-shaped compared to healthy cells."

F-actin was discovered in 1887, but despite the more than 18,000 actin-related studies in scientific literature, biologists have struggled to unlock some of its secrets. For example, F-actin is a polymer made of many smaller proteins called monomers. These building blocks, which are called G-actin, self-assemble end to end to form F-actin. But the self-assembly process is so efficient that scientists have been unable to see what happens when the first two or three monomers come together to form the nucleus of a filament. The F-actin filaments inside cells are constantly being built, torn apart and rebuilt.

"Nucleation is critical for this continual building and rebuilding," said BCM biochemist and study co-author Qinghua Wang. "For healthy cells, nucleation is the starting place for robust shape. For unhealthy cells, like cancer, nucleation processes may play a crucial role in unregulated growth. That's one reason we want to better understand nucleation."



This image shows the 3-D structure of the F-actin nucleus. The core of the two actin monomers contained in the nucleus are depicted in green and purple. Credit: X. Chen/BCM

In 2008, Ma and Wang asked Xiaorui Chen, a graduate student in BCM's Structural and Computational Biology and Molecular Biophysics program, to undertake the task of using x-ray crystallography to determine the structure of the actin nucleus. Her initial attempts failed, but the team finally hit upon the winning idea of creating two mutant versions of G-actin that could nucleate but not polymerize.

Native G-actin binds with one neighbor on top and one on bottom, and this top-bottom, end-to-end binding pattern is the key to forming long F-actin polymers. To foster nucleation without polymerization, Chen created two mutant versions of G-actin. One mutant could bind normally on top but not on bottom, and the other could bind normally on bottom but not on top.

"This dual-mutant strategy was the key," said Chen, who is now a postdoctoral researcher at BCM. "After that, we had to overcome problems related to forming and growing the crystal samples needed for crystallography."

Chen used a two-stage process to prepare the crystals. She first used high levels of super-saturation to spur initial crystal formation and then used a process called seeding to transfer the newly formed crystals to another medium where they could grow large enough for examination.

Once the crystals were prepared, they were analyzed with x-ray diffraction, which revealed the atomic arrangement of each atom in the nucleated, dual-mutant pair. "We believe this dual-mutant arrangement reveals the most critical contacts involved in nucleation," Ma said. "For the first time, we are able to see how actin nucleation begins."

More information: www.cell.com/cell-reports/full … 2211-1247(13)00210-6

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