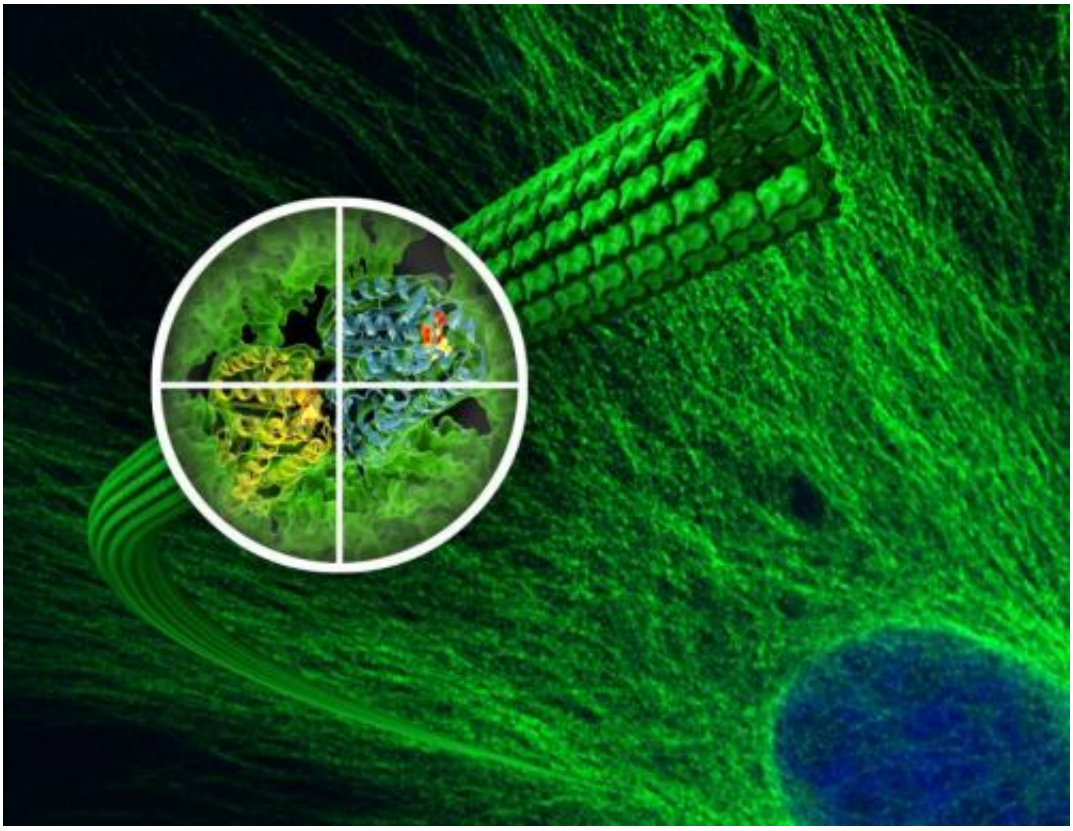
Discovery of how Taxol works could lead to better anticancer drugs

May 22 2014, by Robert Sanders



The most detailed look ever at the assembly and disassembly of microtubules, tiny fibers of tubulin protein that play a crucial role in cell division, provides new insight into the success of the anti-cancer drug Taxol. Credit: Image courtesy of Nogales Lab

University of California, Berkeley, scientists have discovered the extremely subtle effect that the prescription drug Taxol has inside cells

that makes it one of the most widely used anticancer agents in the world.

The details, involving the drug's interference with the normal function of microtubules, part of the cell's skeleton, could help in designing better anticancer drugs, or in improving Taxol and other drugs already known to disrupt the workings of microtubules.

The findings are being reported in the May 22 issue of the journal *Cell*.

"Efforts towards understanding these chemotherapeutics better are very important, because there are some microtubule differences in cancer cells versus normal cells that maybe we can exploit," said principle author Eva Nogales, a biophysicist, UC Berkeley professor of molecular and cell biology and senior faculty scientist at Lawrence Berkeley National Laboratory (LBNL). "We are not there yet, but this is the kind of analysis we need to get there."

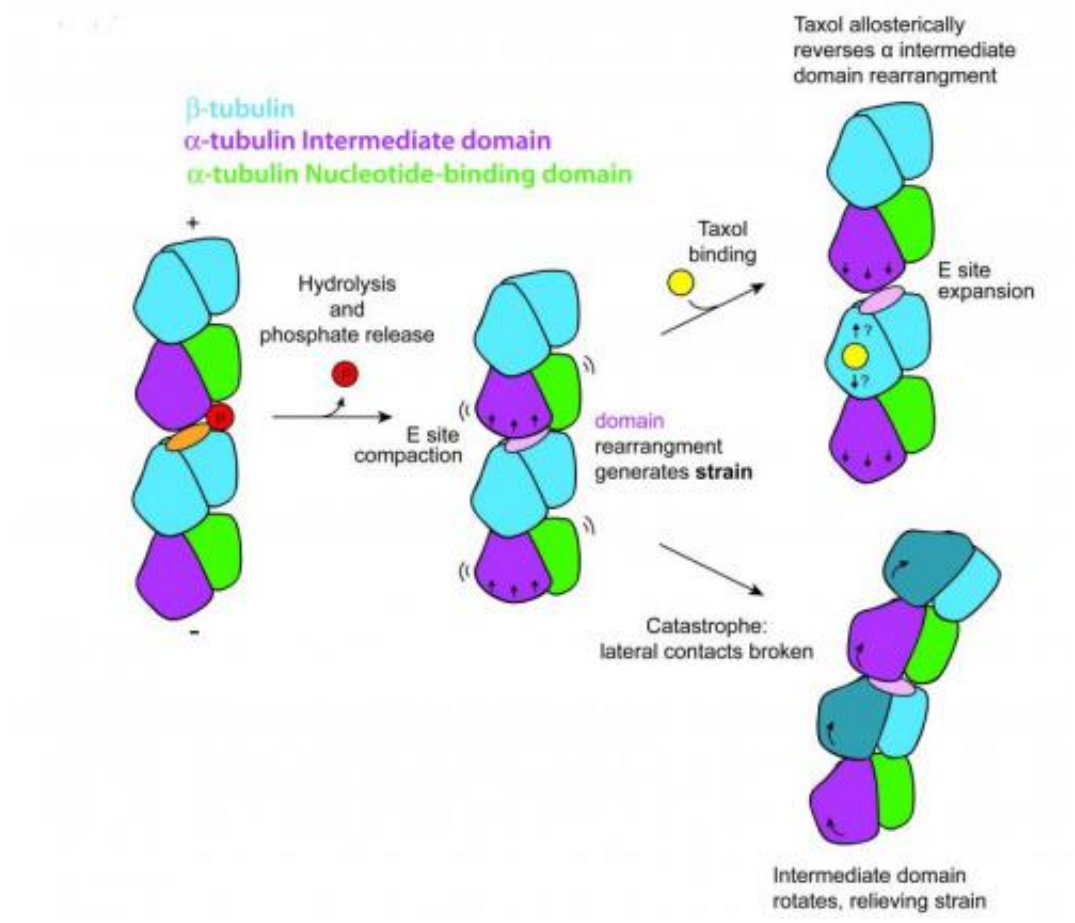
Taxol, originally extracted from the bark of the Pacific yew tree, is one of the mostly commonly used drugs against solid tumors, and is a front-line drug for treating ovarian and advanced breast cancer. The drug is known to bind to microtubules and essentially freeze them in place, which prevents them from separating the chromosomes when a cell divides. This kills dividing cells, in particular cancer cells, which are known for rapid proliferation.

Nogales, a Howard Hughes Medical Institute investigator, has worked on microtubules since she was a doctoral student in England in the early '90s, using techniques such as X-ray scattering and cryoelectron microscopy to study how Taxol and other anticancer agents affect microtubules. Later, during her postdoctoral work at LBNL with Ken Downing, she was the first to discover exactly where Taxol binds the basic building block, called tubulin, of the microtubule polymer.

Microtubules are the cell's skeleton

Work by many scientists around the world has shown the microtubule network inside cells, called the cytoskeleton, to be very different from rigid animal skeletons. Microtubules are polymer filaments that constantly grow and shrink, and in doing so push and pull things around the cell, including the chromosomes. Scientists call this dynamic instability. The microtubules also provide a highway for transporting the cell's organelles and other packages around the cell.

Tubulin, the basic structural unit of the microtubule, is a complex of two proteins – alpha and beta tubulin. Tubulin units stack one atop another to form strips that align with other strips and then zip up to form a hollow tube, the microtubule.



GTP hydrolysis of tubulin protein releases a phosphate, causing compaction of the E-site and rearrangement of the α-tubulin monomer that generates a destabilizing strain on the structure. Taxol binding reverses the E-site compaction and α-tubulin rearrangement. Credit: Image courtesy of Nogales Lab

"Tubulin, the cytoskeletal protein that self-assembles into microtubules, is absolutely essential for the life of every eukaryotic cell, which is why it has become a major target of anticancer agents," Nogales said. "It's amazing how microtubules probe and try new things almost at random, but there is a level of control built into the cell that ultimately makes sense of this chaos, and the cell survives and prospers."

Microtubules grow from their free end at about 1 micron per minute by continually adding more tubulin (around 20 tubulin molecules per second). But if they stop growing, they rapidly peel apart like the skin of a banana, releasing tubulin for recycling into other microtubules. This peeling, or depolymerization, takes place at up to 15 microns per minute, or about 300 tubulin molecules falling off per second, Nogales said.

Microtubules are like compressed springs

Nogales has now discovered why microtubules peel apart so rapidly. When they assemble, the strips of tubulin are put under intense strain, but prevented from bending and pulling apart by the growing cap of tubulin on the end. Once growing stops and that cap disappears, the restrained tension rips the microtubule apart.

The tension is created when the tubulin complex, which has a small energy molecule called GTP (guanosine triphosphate) attached, becomes

hydrolyzed and the GTP turns into GDP (guanosine diphosphate). This chemical reaction compacts the alpha and beta subunits, much like compacted vertebrae, keeping the tubulin stack under tension as long as the microtubule is growing at its end.

"It had been proposed that tubulin had to be constrained, but no one had proved it," Nogales said. "What we have seen is that as GTP hydrolysis happens, the tubulin structure gets stuck in a strained state, like a compressed spring. The end subunits are holding the whole thing together."

When growth stops, the tension is unleashed, and the strips peel apart rapidly.

"This work represents a major step forward on a problem with a long history," wrote Tim Mitchison in a commentary in the same issue of *Cell*. Mitchison, a Harvard University professor of systems biology, was the first to show the importance of GTP hydrolysis in destabilizing microtubules. The model proposed by Nogales and her team, he added, "provides our first glimpse into (the) destabilization mechanism."

Nogales also found that Taxol inserts itself into the tubulin protein and prevents compaction of the alpha and beta subunits, so that no tension builds up. As a result, even if the microtubule stops growing, it remains intact, basically frozen in place, unable to peel apart, or depolymerize, and carry out its normal function.

"Taxol reverses the effects of GTP hydrolysis," she said.

Nogales and her team discovered these structural changes by pushing the limits of cryoelectron microscopy, a technique in which samples are frozen and probed with a high-powered electron beam. They have now achieved a resolution sufficient to see details smaller than 5 angstroms

(one-tenth of a nanometer) across, which is about the size of five hydrogen atoms. While most information to date about the structure of tubulin inside the microtubule has come from the study of artificial, flat sheets of aligned strips of tubulin, Nogales was able to probe three-dimensional microtubules frozen into their natural state, with and without Taxol bound to tubulin. This comparison clearly showed the effect Taxol has on microtubule structure.

More information: High resolution microtubule structures reveal the structural transitions in tubulin upon GTP hydrolysis, *Cell*, 2014.

Provided by University of California - Berkeley