Researchers model genome copying-collating steps during cell division

May 23 2011

Researchers from Virginia Tech and Oxford University have proposed a novel molecular mechanism for the living cell's remarkable ability to detect the alignment of replicated chromosomes on the mitotic spindle in the final phase of the cell division cycle. This checkpoint mechanism prevents mistakes in the cell division process that could damage dividing cells and the organism they inhabit.

John Tyson, University Distinguished Professor of Biological Sciences in the College of Science at Virginia Tech, and Bela Novak, professor of integrative systems biology at Oxford University, have been using mathematical models for many years to study the checkpoints that regulate irreversible progression through the cell cycle. Their latest modeling effort, on the chromosome alignment checkpoint, is published in the online early edition of the Proceedings of the National Academy of Sciences (PNAS) the week of May 23 in the article, "System-level feedbacks make the anaphase switch irreversible," with coauthors Enuo He and Orsolya Kapuy of the Centre for Integrative Systems Biology at the University of Oxford; Raquel A. Oliveira of the Department of Biochemistry, University of Oxford; and Frank Uhlmann of the Chromosome Segregation Laboratory, Cancer Research UK, London.

The article provides theoretical and experimental evidence that bistability of the checkpoint machinery ensures irreversibility of the metaphase-anaphase transition.

The most important goal of the cell division cycle is to make a new copy

of each of the cell's DNA molecules and then to separate these identical molecules, called sister chromatids, to the two new cells so that each cell gets one and only one copy of each DNA molecule. "Think of it like copying the pages of a book," said Tyson, "where the two copies of each page are stuck together when they come out of the copy machine, and then putting the copies through a collating machine that pulls apart the identical pages, placing one copy in a stack to the right and the other copy in a stack to the left. The DNA synthesis phase of the cell cycle, called S phase, is the copy machine, and mitosis, called M phase, is the collating machine," he said.

"The copying and collating machines must be carefully monitored by the cell, because mistakes in replicating the DNA molecules or partitioning the sister chromatids to the new cells can be fatal. It is the job of molecular 'surveillance mechanisms' to look for mistakes and correct them," said Tyson. "If a mistake is found, then further progress through the cell division cycle must be blocked until the problem can be corrected."

These block-points are called checkpoints. Once a cell has passed one of these checkpoints, it may not back up to an earlier phase of the cell division cycle; it must proceed to the next phase. In this sense, the checkpoint transitions are said to be irreversible.

In 1993, Tyson and Novak proposed that the transition into mitosis (the G2/M transition) is irreversible because it is controlled by a molecular toggle switch.

"A mechanical toggle switch, like an old-fashioned light switch, has two states: off and on. To turn the light on, the lever must be pushed up, above the mid-point, before the switch flips on," said Tyson. "Once the light is turned on, it stays on; the transition is irreversible in the sense that this same switch will not mistakenly turn the light off of its own

accord. To turn the light off, the lever must be pushed down, below the mid-point, before the switch flips off. When the lever is in the central position, the lights may be either on or off, depending on which direction the lever is moving. In the central position, the switch is 'bistable'. The irreversible transition points (where the switch flips) lie above and below the central, bistable area."

This connection between bistability and irreversible transitions extends to the tiny molecular switches in the cell division cycle, as proposed by Novak and Tyson in 1993. In 2003, their prediction was confirmed experimentally by Jill Sible, associate professor of biological sciences at Virginia Tech, and her research group, and by Jim Ferrell's group at Stanford University. Since then bistability and irreversibility have been confirmed at two other cell cycle transitions, but the 'metaphase-anaphase transition' remained a puzzle.

Tyson explained that metaphase is the critical step in the collating machine, when the glued-together pages (the sister chromatids) are all lined up in the central zone (the metaphase plate) with one page attached by a string (microtubule) coming from the left side of the cell and the other page attached by a string coming from the right side of the cell. "As the cell leaves metaphase and enters anaphase, the glue is dissolved and the pages are pulled apart by the strings to the stacks on the left and right. In this fashion, each stack (each daughter nucleus) gets one and only one copy of each page (each chromatid)."

There is a surveillance mechanism for chromosome alignment that makes sure that none of the glue is dissolved until every one of the glued-together sister-chromatid pairs are properly aligned on the metaphase plate, Tyson said. "Human cells have 46 pairs of sister chromatids. Even if 45 of the 46 pairs are properly aligned, the cell may not pass the metaphase checkpoint. But as soon as the 46th pair comes into alignment, the checkpoint is rapidly lifted, the glue is dissolved, and the

cell moves on to the next stage of the division process. The transition is irreversible in the sense that once the glue is dissolved and the pages are separated, the cell cannot easily return to the pre-metaphase stage. It must go on to divide and start again at the beginning of a new cell cycle."

The theoreticians attribute bistability of the metaphase checkpoint and irreversibility of the metaphase-anaphase transition to two positive feedback loops in the molecular interactions that comprise the surveillance mechanism. The experimentalists (Oliveira and Uhlmann) have shown that, if one of these feedback loops is broken by mutations, then bistability is lost and the transition becomes reversible. The nature of the second feedback loop is still controversial and the subject of ongoing experimental studies.

"Understanding these control mechanisms is important," said Tyson, "because mistakes in partitioning chromosomes at anaphase are the root cause of many human maladies, like birth defects and cancer."

Provided by Virginia Tech