Scientists identify key decision-point at which cells with broken DNA repair themselves or die

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When cells undergo potentially catastrophic damage, for example as a result of exposure to ionizing radiation, they must make a decision: either to fix the damage or program themselves for death, a process called apoptosis.

It's a stark decision that is as mysterious as it is remarkable, involving what might be described metaphorically as a network of internal alarms that detect damage to DNA packed tightly in the cell nucleus. In intricate ways, cells orchestrate a response to signals from such resident sensors when they are triggered by exposure to radiation or other toxic processes, which if unchecked can cause genes to mutate and cancerous tumors to begin forming.

A team of biologists led by Professor Nicholas Tonks, Ph.D., F.R.S., of Cold Spring Harbor Laboratory (CSHL), this week revealed results of experiments suggesting at least one way in which cascades of intracellular signals are regulated at what they call a decision point - as cells decide whether to repair broken DNA strands or commit suicide following DNA damage.

In a report published online ahead of print in the *Journal of Biological Chemistry*, Tonks and colleagues identify a protein with the unlikely name Eyes Absent, or EYA, as performing a critical role in setting the damage-repair machinery in motion. Engaged within the larger context

of a complex signaling cascade within the cell, EYA regulates the formation of specialized microenvironments on DNA, called gamma-H2A.X foci, which allow the cell to summon repair enzymes to the site of broken DNA strands. The team's experiments, conducted by Navasona Krishnan, Ph.D., of the Tonks lab, showed that when Eyes Absent was not present in damaged cells grown in culture, such foci were not formed and the cells went the route of apoptosis—they programmed themselves to die.

'A lovely moment' of discovery

Tonks says the finding is "a powerful example of multiple lines of research and different kinds of expertise coming together" in what he describes as "a lovely moment" of synthesis and discovery. An important collaborator in the work, C. David Allis, Ph.D., of the Rockefeller University, had recently published a paper in Nature describing a critical component of the DNA damage-repair signaling cascade. The Tonks-Allis collaboration, along with contributions from Seung Jun Kim, Ph.D., a protein crystallographer from the Korea Research Institute of Bioscience and Biotechnology, led to the assembly of a puzzle from pieces whose precise relation was not previously understood.

The parts of the puzzle include a protein called H2A.X, one of a species of proteins called histones that form structures around which DNA is "spooled" for dense packing in the cell nucleus. "David Allis showed us that this particular histone, which is found at those critical decision points called gamma-H2A.X foci, has phosphate groups added to its structure at particular points at the end of the protein. David demonstrated that another kind of protein --a kinase called WSTF -- attaches phosphate to critical site, a tyrosine residue, at the extreme end of the protein," Tonks explains..

"I was interested in the fact that when there is damage to double-

stranded DNA - catastrophic damage, such as when the strand breaks in two - the phosphate molecule placed at the decision point by WSTF has to be removed in order for the cell to send out signals for DNA-repair enzymes to come to the scene."

The removal of phosphate groups from proteins is accomplished by a family of enzymes called phosphatases - the focus of much research in Tonks' CSHL laboratory. Tonks is well known for having characterized the first of what has come to be understood as a large superfamily of protein tyrosine phosphatases, or PTPs - enzymes that specifically remove phosphate molecules from amino acid residues called tyrosines. This function is critical in regulating cellular signaling in normal and disease conditions. In effect, important kinds of cellular signals can't be sent without helper enzymes like PTPs that remove phosphate molecules from specific locations, and kinase enzymes that perform the reverse role - that of adding phosphates.

Another vital piece of the puzzle in the current work involves Dr. Kim, who recently spent 15 months in the Tonks lab at CSHL, working on growing crystals of protein phosphatases to determine their structure, including that oddly named protein, Eyes Absent. Why that one? As Tonks explains, "EYA is known from work on the fruit fly, or Drosophila, to be a very unusual protein: it's the only one we know of that acts in the cell nucleus to regulate genes - what we call a transcription factor - while, in other contexts, is also known to act as a phosphatase - that is, it can remove phosphate groups from other proteins."

The role of Eyes Absent in DNA damage repair

EYA was shown to be a phosphatase by three separate groups in 2003. Although it was thought to be a PTP - i.e., it was thought to take phosphate groups off tyrosine residues in proteins - the identity of its

target proteins in the cell was unknown. Kim's structure was important because it revealed a particular distribution of charged residues on the surface of the protein that suggested to Tonks the possibility that basic proteins, such as histone H2A.X, may be one such critical substrate.

This led to the experiments in which Tonks' team showed that Eyes Absent was in fact the protein that removed the critical phosphate group from the end of histone H2A.X, thereby allowing the formation of the so-called gamma-H2A.X foci, which set DNA repair in motion when double strands were broken. When EYA was experimentally "knocked out" via a technique called RNA interference, or RNAi, damaged cells with double-stranded DNA breaks did not repair themselves; instead, they simply died -- underwent apoptosis.

As is often the case in science, multiple labs are engaged on related subjects. Results similar to those reported by Tonks and colleagues recently have been obtained in Geoff Rosenfeld's lab at the University of California, San Diego. But there is more work to be done of the subject. It remains unclear if or how the role of Eyes Absent in the DNA repair machinery -- in other words, its role as a tyrosine phosphatase, or remover of phosphate molecules -- is related to its role, in other contexts, as a transcription factor (a regulator of gene expression). It is certainly a curiosity that a protein that can regulate genes in certain contexts can act in others as the fulcrum in a mechanism that repairs damaged genes. Tonks and colleagues expect to explore this in future work.

More information: "Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase Eyes Absent (EYA)" appeared online ahead of print April 7 in the Journal of Biological Chemistry (www.jbc.org/cgi/doi/10.1074/jbc.C900032200). The full citation is: Navasona Krishnan, Dae Gwin Jeong, Suk-kyeong Jung, Seong Eon Ryu,

Andrew Xiao, C. David Allis, Seung Jun Kim, and Nicholas K. Tonks.

Source: Cold Spring Harbor Laboratory (news : web)