Analysis reveals extent of DNA repair army

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Cells have the remarkable ability to keep track of their genetic contents and -- when things go wrong – to step in and repair the damage before cancer or another life-threatening condition develops.

But precisely how cells monitor the integrity of their genomes, identify problems, and intervene to repair broken or miscoded DNA has been one of nature's closely held secrets. Now, however, a report in the journal *Science* describes a new database developed by a team of researchers from the Howard Hughes Medical Institute at Harvard Medical School that is providing the first detailed portrait of the army of more than 700 proteins that helps maintain DNA's integrity.

"The generation of this database is changing the way we think about the DNA damage response," explained HHMI investigator Stephen J. Elledge of Harvard, the senior author of the new study published on May 25, 2007, in *Science*. "Our work paints a broader landscape of this critical response that helps cells keep their genomes intact."

The DNA damage response is a routine event in the life of any cell. Stress caused by environmental factors such as exposure to ultraviolet light, ionizing radiation or other environmental phenomena can cause DNA to break apart or rearrange its nucleotide base pairs in unhealthy ways. If such mutations are left unchecked, they can accumulate over time and lead, ultimately, to cancer or diabetes.

Elledge likened the DNA damage response to a command and control center: "It sends out sensors and sends out an alarm to start activating

different repair pathways."

Elledge explained that two critical enzymes, known in scientific shorthand as ATM and ATR, act like sensors to detect trouble and initiate the DNA damage response by engaging the cell's molecular repair apparatus. By looking to see how ATM and ATR reacted to damage in cells, Elledge's group found that a small molecular army -- more than 700 different proteins -- is called into physiological action when the cell's DNA is in need of repair.

Elledge's group studied human cells in culture and mapped their response to ionizing radiation and ultraviolet light. Specifically, the group looked to see which proteins in the cell were chemically altered by the enzymes ATM and ATR, finding 900 sites on 700 proteins that changed in response to DNA damage. The discovery that so many proteins are involved in the process, Elledge said, was a big surprise.

"The results of this study illustrate the extraordinarily broad landscape of the DNA damage response, which extends far beyond what was anticipated from previous studies," he said.

The revelation that so many proteins are involved in repairing faulty DNA opens a molecular frontier that promises insight into a spectrum of diseases. For example, in a companion paper, also published this week in Science, Elledge's group used the new DNA repair database to identify two proteins critical to recruiting the BRCA1 gene to sites of DNA damage. The BRCA1 gene is known to protect against breast and ovarian cancer by suppressing tumors.

"Now we have a better idea of how the BRCA1 protein is being targeted to DNA damage sites," Elledge said, and this seems to run a pretty big part of the BRCA1 program."

The proteins, known as Abraxas and RAP80, bind to the BRCA1 protein and form a complex that governs three essential modes of DNA damage control: damage resistance, genetic checkpoints that constrain cell proliferation, and DNA repair. There are three variants of this BRCA1 complex and one is mediated by Abraxas and RAP80, providing potentially different windows into the protective nature of the gene.

"We have to stop thinking about BRCA1 as a single entity. There are three complexes and which complex is doing what" That's what needs to get figured out," Elledge said.

He noted that simply knowing that BRCA1 comes in three distinct flavors gives researchers the chance to sort out the role of each in the DNA damage response and the onset of tumors.

An intriguing offshoot of the findings, Elledge said, is that they provide a potential lead into the role of estrogen in breast cancer.

"RAP80 also binds the estrogen receptor and may have roles in signaling through the estrogen pathway," he said. "I think this may be a big clue about how BRCA1 has breast specificity for tumorigenesis."

That is important, Elledge explained, because one of the big unanswered questions about BRCA1 is why it acts to suppress only some forms of cancer: The gene is "common in (all) cells, so why breast cancer and not some other kind of cancer""

Exploring the substrate domains of the ATM and ATR enzymes, Elledge added, is certain to yield deeper insight into numerous conditions mediated by faulty DNA repair mechanisms. For instance, using the new database produced by his group, Elledge and his colleagues identified a protein made by the FANCI gene, implicated in Fanconi anemia, a developmental and cancer-predisposition syndrome. The syndrome is

caused by faults in genes that control the ability to mend broken DNA.

These findings were published on April 20, 2007, in the journal Cell.

The point, Elledge argued, is that the new picture of the vast and sophisticated protein network that underlies a cell's response to DNA damage has the potential to unmask the molecular interplay at the root of many diseases and conditions.

"These things are really important," he said. "This is how things get coordinated and how the different repair pathways are activated. Every time you duplicate a cell, there are enough problems that this mechanism is called into action."

Source: Howard Hughes Medical Institute