Regulating the nuclear architecture of the cell

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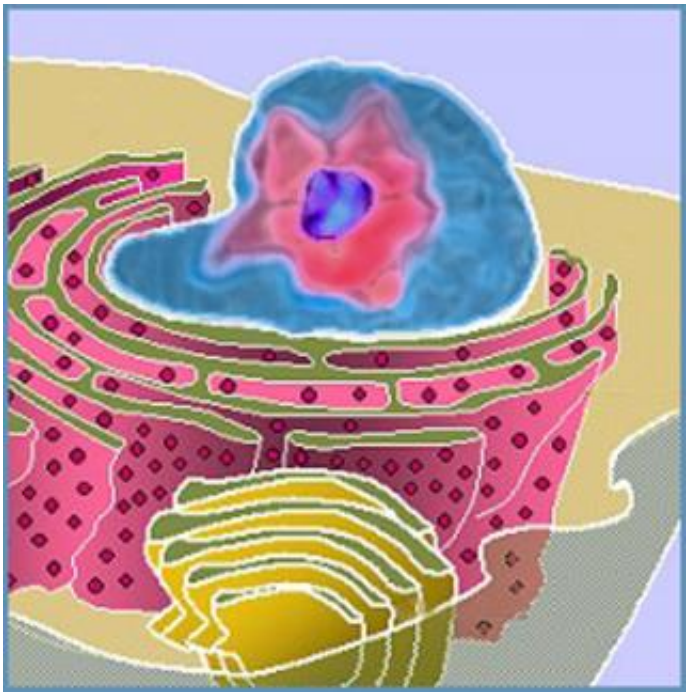

The nucleolus (dark blue) resides within the cell nucleus, surrounded by heterochromatin. Berkeley Lab researchers have discovered the molecular pathways that regulate its organization. Credit: Lawrence Berkeley National Laboratory

An organelle called the nucleolus resides deep within the cell nucleus and performs one of the cell's most critical functions: it manufactures ribosomes, the molecular machines that convert the genetic information carried by messenger RNA into proteins that do the work of life.

Gary Karpen and Jamy Peng, researchers in the Life Sciences Division

of the Department of Energy's Lawrence Berkeley National Laboratory, have now discovered two pathways that regulate the organization of the nucleolus and other features of nuclear architecture, maintaining genome stability in the fruit fly Drosophila melanogaster. Their results are published in *Nature Cell Biology* and are now available online to subscribers.

Because much of the genome of Drosophila is shared with human beings, learning how nuclear organization is controlled in the fruit fly can apply to human disorders like birth defects and cancer. The organization of structures in the cell's nucleus has profound effects on such essential functions as how and when genes are expressed. When regulation fails, genome aberrations accumulate, including repeated sequences of DNA or even entire extra chromosomes.

"Our project continues to point to an understanding of genome stability in humans," says Karpen, who heads Berkeley Lab's Department of Genome and Computational Biology.

The epigenetics of heterochromatin

Controlling functions of the cell and organism through nuclear architecture and spatial rearrangements is known as epigenetics from the Greek for "on, over, or at" the genes, instead of by the DNA sequence. The chromosomal material known as heterochromatin mediates gene silencing, chromosome inheritance, and other processes. Karpen and Peng have identified the molecular pathways that regulate two of heterochromatin's important functions.

One of these is control of repeated DNA sequences in and outside the heterochromatin. The other is the organization and structure of the nucleolus, the ribosome factory, which is situated at the specific site in the heterochromatin where ribosomal DNA -- consisting of large genes,

repeated 300 to 400 times -- codes for the production of the RNA from which ribosomes are built.

"This work on pathways that control the organization of the nucleolus is the first to be published that deals with an organelle," says Karpen. "Even though the gross organization of chromosomes and other nuclear elements is well known in cell biology, learning about the regulation of nuclear architecture is in its early stages."

The most striking feature of any nucleus is its chromosomes. These are made of chromatin, which combines DNA with a set of proteins known as histones; four similar histones join together to form a cylindrical spool around which the DNA wraps. Each of these bundles is called a nucleosome, and many nucleosomes are bound together by the continuing strand of DNA, which forms a string of beads that further coils to form one of two kinds of chromatin, either euchromatin or heterochromatin.

Most genes reside in euchromatin, which is of relatively low density and where the DNA is more accessible to the machinery of gene transcription. By contrast, heterochromatin is dense and contains relatively few genes; most of the DNA in heterochromatin, including numerous short repeated sequences, does not code for proteins.

Heterochromatin is typically found at the ends of a chromosome, where it abuts the telomeres -- chromatin structures best known for limiting, by their diminishing length, how many times a cell can replicate. Heterochromatin also flanks the centromere in the central region of the chromosome, the chromatin structure that plays a crucial role in chromosome segregation during cell division. What other functions abundant heterochromatin may perform are still an open question.

Even epigenetics ultimately has its roots in the genes; the researchers'

first step was to identify which genes affect organization of the heterochromatin, then to identify the proteins expressed by these genes, and finally to learn how they act on the chromosomal material.

Steps along the path

Several genes previously identified in Drosophila as having a role in the suppression of gene expression, known as suppressors of variegation, were named Su(vars). The gene family known as Su(var)3-9 codes for proteins that chemically modify certain histones in the nucleosomes.

Su(var)3-9 proteins attach methyl groups to the histone labeled H3 at its ninth amino acid residue, which is a lysine. In the histone code the methylation target is known as H3K9, where K stands for lysine. Methylation of chromatin causes it to condense.

Another Su(var) gene, Su(var)2-5, makes a protein called HP1 that binds methylated H3K9 and Su(var)3-9 proteins; the effect is to further close up the chromatin, silencing genes by making access to DNA more difficult.

Other genes called E(var)s act to enhance variegation in Drosophila. One way they do this is by acetylation of the H3 histone's fourth amino acid, also a lysine. The effect is to open the chromatin at that site, making the DNA accessible.

"Acetylating H3K4 and demethylating H3K9 can activate a gene; deacetylating H3K4 and methylating H3K9 can silence it," Karpen explains. "Multiply modified at various locations, these histones are a major factor in changing the functions of chromatin, independent of DNA sequence."

Karpen and Peng studied fruit flies with mutant Su(var)3-9 genes, flies

that could not produce HP1 proteins or the proteins necessary to methylate H3K9. The results were dramatic. Because the ribosomal DNA genes could not be silenced, rDNA proliferated. These and other repeated DNAs became dispersed and disorganized throughout the cell nucleus. Instead of a single well-formed nucleolus where genes for ribosomal DNA were normally located in the heterochromatin, multiple nucleoli appeared in the nucleus.

Mutations in another pathway, that of RNA interference (RNAi), produced related results. One way RNAi works is by unleashing the Dicer-2 enzyme to chop strands of RNA into short lengths, which can degrade messenger RNA and prevent gene expression.

Karpen explains that "the RNAi pathway is a sort of immune system against viral DNA and transposons." Transposons are pieces of DNA that can "jump" to different locations in the genome, either directly or by copying themselves, inserting themselves in new sequences where they can cause mutations.

"Short repetitive pieces of DNA that keep copying themselves could conceivably expand and take over the whole genome," says Karpen, "so some balance must be achieved, between the need for replication processes on the one hand and the need to repress or control or regulate those processes on the other."

Since DNA pieces that copy themselves must use RNA intermediates, these can be disabled by RNA interference. Transposons are also silenced by accumulating in the transcriptionally-silent heterochromatin, which Karpen calls "a graveyard of transposons."

While the gene for the Dicer-2 enzyme, dcr-2, is a key player in the RNAi pathway, the RNAi process also targets H3K9 methylation. Sure enough, Karpen and Peng found that Drosophila with mutant dcr-2 genes

showed multiple nucleoli and other symptoms of heterochromatin deregulation, just as Su(var)3-9 mutants did.

The road to regulation

One effect of both Su(var)3-9 and RNAi mutations was to increase the amount of extrachromosomal DNA, typically small loops of repeating sequences, in cell nuclei. This observation led Karpen and Peng to propose a specific mechanism by which H3K9 methylation and RNAi pathways regulate nuclear organization.

When the H3K9 methylation pathway is functioning properly, heterochromatin remains condensed and a single nucleolus forms around the ribosomal DNA. But lack of H3K9 methylation -- whether from a mutation in Su(var)3-9; or in the gene that codes for the HP1 protein; or a mutation that disables RNA interference -- allows the heterochromatin to open up. The decondensed, repeated DNAs are then free to interact; DNA recombination and repair processes excise the DNA from the chromosome to form extrachromosomal DNA. If these extrachromosomal loops consist of ribosomal DNA, they further accelerate the production of misplaced nucleoli.

Curiously, says Karpen, this chaotic state of affairs is not, by itself, fatal to the organism. "I wondered why the flies with mutations in these important pathways didn't die. But replication and cell division checkpoints slow down the cell cycle when damaged DNA is detected, and DNA repair mechanisms move in to fix the damage." However, if these replication checkpoints are crippled in flies that also have mutations in the H3K9 methylation and RNAi pathways, Karpen says, genome aberrations accumulate "and they do die."

Says Karpen, "These findings have broad implications for genome stability. We plan to investigate whether the H3K9 and RNAi pathways

we've identified in Drosophila play the same role in humans; if so, misregulation of heterochromatin could be one cause of the rampant genome instability observed in cancer. Beyond these specific pathways, we're interested in the larger question of how such pathways affect the organization of the chromosomes in the cell nucleus."

Source: Lawrence Berkeley National Laboratory