Three of the most crucial mechanisms required for cellular health are endocytosis to transport signaling molecules from the surface of cells into the cytoplasm, autophagy for conversions and disassembly to optimize space within cells and the third one is for nuclear restorer factors (NRFs) that enables interaction between the two chromosomes that exist in cells.

Step by step modeling explanations for endocytosis and autophagy are provided elsewhere on the MCFIP website. The following is provided for use as talking points for NRF activity with interested parties.

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In 2013 our modeling of NRF activities (i.e. CLAMP) identified the epigenetic mechanism as selenium - zinc based on formation from byproducts of hydrogen sulfide using the following process:

http://www.mcfip.net/upload/Cell%20Surface%20Signaling%20Molecule%20Formation%207-2017.pdf

The process explains how cell surface signaling molecules are formed and, as needed, they can be imported by endocytosis from the cell surface to the cytoplasm.

Refer to the following for endocytosis:
http://www.mcfip.net/upload/Endocytosis%20Modeling%204-30-17.pdf

Our recent discovery for the interactions between byproducts of multiple gasotransmitters prompted us to revisit the 2013 model and create the verifiable explanation that is outlined in the attached.

Fruit fly protein dual duties may make it model for studies of protein function in context

August 28, 2017

An essential fruit fly protein called CLAMP may help biologists answer the key question of how the same protein can manage to coordinate two completely different processes on distinct chromosomes in the same cell.

New research on a crucial protein in fruit flies provides a clear model for a fundamental question in biology that's significant for drug development in particular: What influences the exact same protein to coordinate a vital molecular process on one chromosome but an entirely different one on another chromosome?

The new study concerns the recently discovered protein CLAMP. Previously, scientists at Brown University had identified CLAMP as the linchpin in the process by which cells in males doubly express their single X chromosome to achieve genetic parity with females, a process necessary for male existence and survival. Now, in a study published in the journal *Genes and Development*, the researchers have identified another role for CLAMP that is equally essential to males and females alike—the protein is responsible for coordinating the process by which the DNA in newly replicating cells of an embryo becomes properly wound up and structured.

"It's really exciting because now we have these two separate chromosomes on which CLAMP does vital jobs," said senior author Erica Larschan. "That sets us up for a compare-and-contrast strategy where we can understand how one protein can function differently in context-specific ways."

That matters, added co-lead author Leila Rieder, a postdoctoral researcher at Brown, because in order for clinical interventions that target key proteins to do more good than harm, they need to be tailored to a specific context. It may be tempting to block or amplify a gene or protein to treat a disease, but without confining the intervention to that one process, it could upset the entirely healthy actions of the same gene or protein in an unrelated process. That could produce potentially devastating side effects.

"One of the biggest fears about using genetics in people is that there are off-target effects," Rieder said. "You don't know when you manipulate a gene if it's going to have a single effect or if it's going to have many effects. We don't understand all the roles that that one manipulation is going to have."

The confirmation of a second life-giving role for CLAMP, Rieder and Larschan said, provides a perfect example of a protein that is essential in two completely different ways in the convenient research model of the fruit fly.

CLAMP goes GAGA

CLAMP binds to DNA all over the fly genome, but it kicks into consequential action when it finds a long series of repeats of the nucleotides GA. In the new study, the scientists found long GA repeats and CLAMP on chromosome 2L at the "histone locus," where a cluster of genes produce the proteins around which DNA gets wound up to fit inside the nucleus. In many organisms, humans included, cells assemble the same cadre of proteins around which they wrap their DNA. Approximately a yard of DNA is present in every microscopic cell, so it is essential that it be tightly packed but still accessible for regulation immediately in a newly fertilized egg.

In a series of experiments, a team at Brown, the University of North Carolina and Massachusetts General Hospital found that in fruit flies, CLAMP is the protein that launches the process of gene regulation that produces histones by recruiting other known regulators. It is among the very first proteins on the scene of the histone locus in a newly fertilized egg and opens up the histone locus for expression by the cell, they found. Experiments in which the team interfered with CLAMP led almost universally to fly eggs failing to hatch.

Foiling CLAMP proved to be so lethal, in fact, that studying its function at all required an experimental ploy that would allow the scientists to manipulate CLAMP while keeping the flies alive. To understand, for example, how CLAMP lures the other histone-related proteins to the histone locus, the Brown team worked with the University of North Carolina collaborators, including co-lead author Kaitlin Koreski, to generate CLAMP mimics that wouldn't interfere with natural CLAMP's DNA binding, but could still attract the other key regulatory proteins that control histone gene regulation.

Same protein, different functions

Larschan and Rieder's new understanding of CLAMP's function at the histone locus now matches their understanding of its function on the X chromosome. But they said they don't yet know exactly what differs about the context of those two chromosomes such that CLAMP, with the same molecular anatomy and bound to the same GA repeats, manages to recruit two completely different groups of proteins to perform separate gene expression tasks.

That's the next step in their research.

"It sets up a paradigm for the future," Larschan said. "There are very few cases—that's what I'm always surprised about when I read the literature—where there are such specific roles at different sites for a single protein. It's a really strong model."

Explore further: GAGA may be the secret of the sexes—at least in insects

More information: Leila E. Rieder et al, Histone locus regulation by the Drosophila dosage compensation adaptor protein CLAMP, *Genes & Development* (2017). **DOI: 10.1101/gad.300855.117**

Journal reference: Genes & Development

Read more at: https://phys.org/news/2017-08-fruit-protein-dual-duties-function.html#jCp

MCFIP – Modeling of the epigenome that applies homeostasis to bodily substances and systems addresses activities that are currently unknown in biomedical research. That being said, because our hypotheses for bodily functions that represent activities that are "in uncharted waters," we must be cautious when putting forth opinions based solely on empirical evidence. Simply, we make such opinions and observations to provide a foundation from which biomedical research can expand and enhance our models that can be verified using our explicit and replicable processes for discoveries we have made thus far.

In keeping with the aforementioned statement, our modeling of the zinc finger based nuclear restorer factors 1 – 3, provides numerous parallels with CLAMP as described in the following article; including but not limited to impacting the X chromosome. In the latter case, we hypothesize that the NRF signaling molecules provide interface between the X and Y chromosomes that reside in different organelles in the cell; i.e. the nucleus and the mitochondria.

It should be noted that early modeling for the source of NRFs had identified them as being byproducts of IL-5 with hydrogen sulfide as the gasotransmitter. Subsequent findings identified byproducts of multiple gasotransmitters (i.e. hydrogen sulfide, nitric oxide and carbon monoxide) as interfacing/interacting to regulate these critical activities.

Early modeling identified a vast number of chronic diseases attributable to NRF mutation. At that time, we had not realized that imbalances between the gasotransmitters could trigger the high volume of outcomes.

Meet CLAMP: A newly found protein that regulates genes

July 16th, 2013 in Genetics /

(Medical Xpress)—A newly discovered protein, found in many species, turns out to be the missing link that allows a key regulatory complex to find and operate on the lone X chromosome of male fruit flies, bringing them to parity with females. Called CLAMP, the protein provides a model of how such regulatory protein complexes find their chromosome targets.

They say a good man is hard to find. Were it not for a newly discovered protein, the X chromosome of a male fruit fly could never be found by a gene-regulating complex that male flies need to develop and survive. And that case is just one example of what the new finding means. More generally, the research provides biologists with a model of how proteins that govern gene transcription find their targets on chromosomes, a process that's essential to healthy cell function and sometimes implicated in disease.

The new protein, dubbed CLAMP by the Brown University scientists who led the discovery, is found in many species including humans. In fly embryos it turns out to be the missing link that brings together the X chromosome and the transcription complex MSL, which doubles the expression of the chromosome. That process, called dosage compensation, brings male flies up to parity with females who have two X chromosomes (in mammals, a similar process downgrades one of the female Xs to ensure parity). In fact, MSL stands for "male-specific lethal" because without it, and without CLAMP, the male flies would die.

Scientists have long puzzled over how MSL and the X chromosome came together, said Erica Larschan, assistant professor of biology in the Department of Molecular Biology, Cellular Biology and Biochemistry and corresponding author of the study published online July 15 in the journal *Genes and Development*. In fact, she said, they've lacked that understanding about many such interactions in which regulatory complexes govern the expression of genes in chromosomes.

"This is the last step of these signaling pathways that make the ultimate regulatory decision about whether you are going to turn on a gene or keep it off at a particular time," Larschan said. "It's exciting because this protein has never been studied before."

In the new paper, Larschan, graduate students Marcela Soruco and Jessica Chery, and their team of collaborators describe several experiments that demonstrate how CLAMP binds to key sites on the X chromosome and then brings in MSL to those sites to do its work. They first turned up the protein in a wide sweep of the fly genome published last year. They were looking for possible missing link candidates, but hadn't yet figured out from the more than 100 they found which ones were genuinely promising. That process took years more work.

As they began to look more closely at CLAMP, they recognized that it has seven zinc ion-tipped "fingers" for grabbing, or clamping, onto DNA. They also noticed it also has a configuration elsewhere that seemed made for binding to a large protein complex.

In their experiments, both in flies and on the lab bench, they show that CLAMP binds DNA at specific sites known to be relevant for MSL's interaction with the X chromosome. They also showed that interfering with CLAMP prevents MSL from finding the X chromosome.

Positive feedback loop

Then they found something that amazed them. Rather than acting simply as an intermediate link, CLAMP works together with MSL to create a self-reinforcing feedback loop of activity at the X chromosome.

"That was a really big surprise," Larschan said. "I did those experiments myself. I kept doing it again and again because I was so surprised."

One of the more telling analyses took advantage of the sex-specificity of the MSL complex. The researchers noticed that while CLAMP would bind to the X chromosome in both male and female flies, it would only progress past a certain degree in the males. The difference is that males have MSL and females don't.

What the researchers determined is that as a male fly embryo develops, CLAMP binds to some initial sites on the chromosome. That facilitates the assembly of MSL at the chromosome. MSL then opens up the coiled up DNA to expose more sites for CLAMP binding, which brings in more MSL.

Larschan speculates that the ability to instigate that kind positive feedback loop, perhaps in the future with a synthetic small molecule drug, could prove therapeutic in any diseases where a regulatory complex and its linking protein isn't operating properly at a chromosome.

"You could theoretically maintain those domains if they were misregulated," she said.

In addition to Larschan, Soruco and Chery, other Brown authors on the paper are Alexander Leydon, Arthur Sugden, Karen Goebel, Jessica Feng, and Peng Xia. Other authors are Eric Bishop, Michael Tolstorukov, and Peter Park of Harvard Medical School; and Tervor Siggers, Anastasia Vedenko, and Martha Bulyk of Brigham and Women's Hospital and Harvard.

Provided by Brown University

"Meet CLAMP: A newly found protein that regulates genes." July 16th, 2013. http://medicalxpress.com/news/2013-07-clamp-newly-protein-genes.html