SPRING 2011

Stowers REPORT

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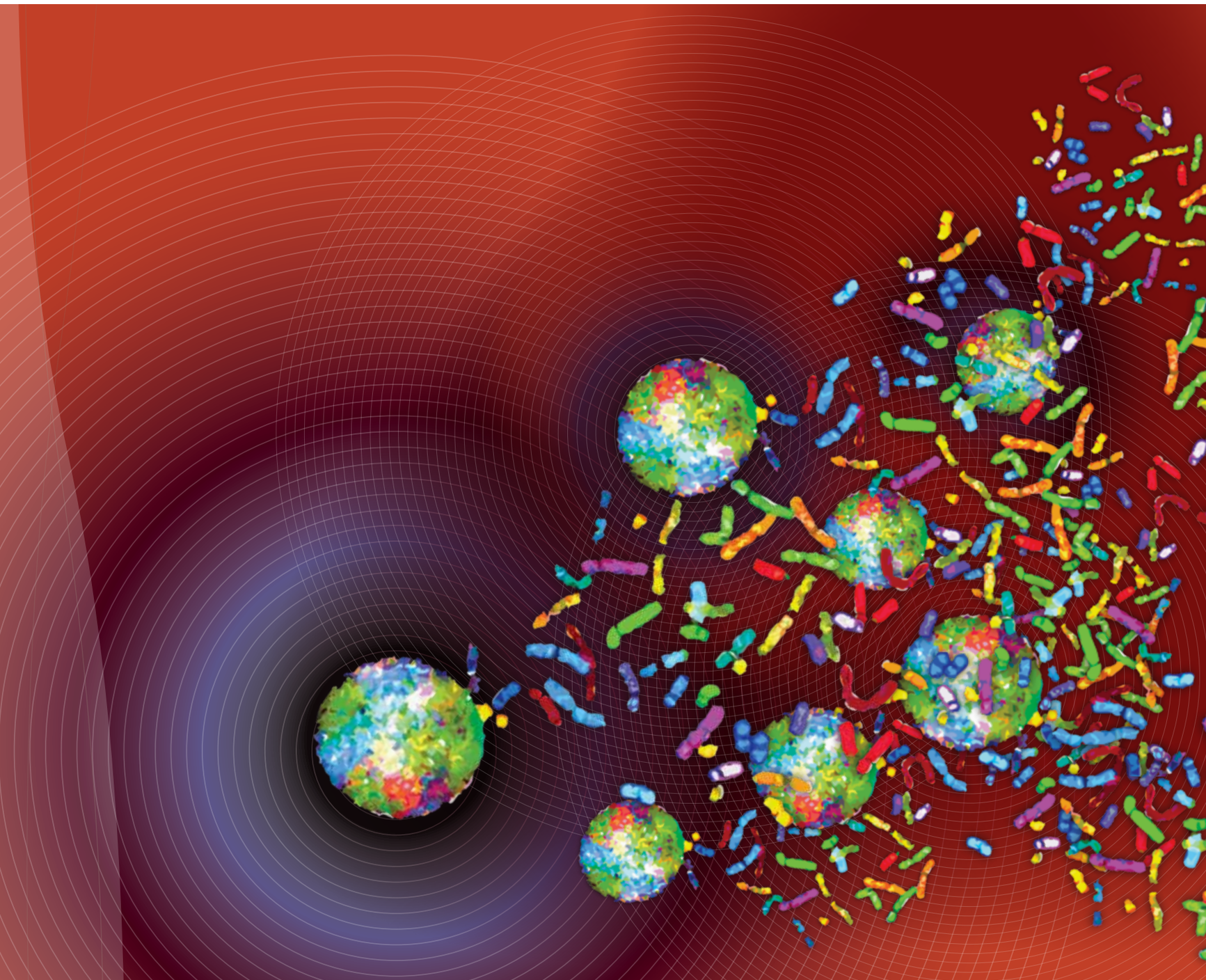

RECENT ADVANCES IN UNDERSTANDING CHROMOSOME DYNAMICS. Page 2

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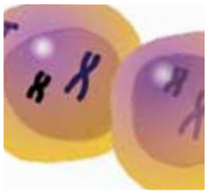

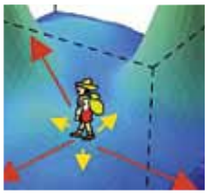

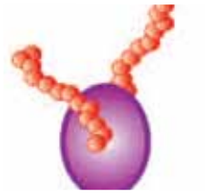

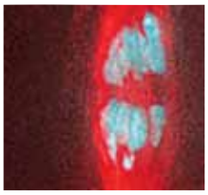

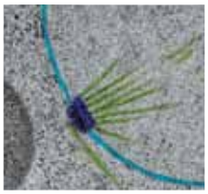

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Cover image: Adapted from an image from the National Human Genome Research Institute, National Institutes of Health, portraying spectral karyotyping, a technique for staining and visualizing chromosomes.

PRESIDENT'S LETTER

BY DAVID CHAO, PH.D., PRESIDENT AND CEO

A typical human cell contains six feet's worth of DNA. In order to fit inside the cell, this DNA is tightly packaged into units called chromosomes. Almost all of the genetic information that makes us human resides in these chromosomes, which undergo numerous changes in order to replicate, segregate, and maintain themselves. The study of these processes defines a field called chromosome dynamics, the theme of this issue of the *Stowers Report*.

Several labs at the Institute focus on chromosome dynamics in organisms as diverse as yeast, fruit flies, mice, and lizards. Their efforts build upon a body of work that spans more than a century. In this letter, I relate some of the early history of chromosome research in order to illustrate principles that are still relevant today.

In 1856, a young English chemistry student, William Perkin, set out to synthesize quinine, a treatment for malaria as well as the main flavor of tonic water. While washing out the residue of a failed experiment, Perkin noticed that the rinsing liquid had turned an intense purple. Perkin had serendipitously discovered mauveine, the first of many synthetic aniline dyes that not only revolutionized the textile industry, but also played an unexpected role in biological research.

In the 1870's, a German biologist, Walther Flemming, used these newly discovered aniline dyes to stain and study cellular structures. After staining fire salamander cells, he observed the nucleus to contain an intensely colored material. He called the material "chromatin," a word he coined from the Greek word for "color." Flemming further observed that chromatin consisted of thread-like structures that separated during cell division. This was one of numerous careful observations by Flemming that together formed a foundation for future work on cell division. Other scientists later observed that the thread-like structures were organized into "chromosomes," a word derived from the Greek word for "colored body."

In the early 1900's, another German biologist, Theodor Boveri, found that the normal development of sea urchin embryos required the presence of all chromosomes. Around the same time, an American biologist and KU alumnus, Walter Sutton, observed that chromosomes in grasshoppers exist in pairs that split during the formation of the egg and sperm. Both scientists independently proposed that chromosomes were the long sought-after molecular carriers of genetic information and linked the previously separate traditions of cell biology and genetics. The proof that genes were indeed carried on chromosomes came from the subsequent studies of an American graduate student, Calvin Bridges. Bridges showed in fruit flies that the improper segregation of genes during the formation of the egg and sperm is the consequence of the irregular segregation of chromosomes.

Over the next century, biologists were successful in characterizing the protein and DNA components that make up chromosomes and used increasingly powerful microscopes to observe the structure and behavior of chromosomes. Many questions remain about chromosome dynamics, and you will read about answers to some of these questions in this issue of the *Stowers Report*.

What can the first century of research on chromosomes teach us about the next century? First, the early history of chromosome research illustrates the significance of serendipity, astute observation, and hypothesis-driven inquiry in basic research. Second, this history shows the importance of selecting an appropriate model organism and developing the proper technology to address a particular research question.

One of the Institute's key tenets is that basic research is most productive when scientists have the freedom and support to pursue interesting questions. With the flexibility of endowment-based funding, scientists at the Institute can focus on the most compelling questions and benefit from the freedom to follow up on serendipitous observations.

The Institute has invested heavily in the technology and expertise to advance work on standard model organisms. However, in the tradition of Flemming, Boveri, and Sutton, the Institute also facilitates work with non-standard model organisms such as the sea anemone, chameleon, whiptail lizard, and planarian. Standard model organisms have the benefit of well-established tools and an existing body of knowledge, but non-standard models enable scientists to pursue complementary approaches to their studies, as well as studies on novel processes of cell division. The Institute's heavy investment in its world-class core facilities such as the Laboratory Animal Services and Reptile and Aquatics facilities reflects our firm belief that access to both standard and non-standard model organisms will be ever more important in the future.

Access to cutting-edge technology remains critical to the success of basic research. However, here the formula for the future departs significantly from what has worked in the past – a good example of this is the microscope. In Flemming, Boveri, and Sutton's era, scientists maintained and often manufactured their own microscopes. Today, microscopes cost more than a five-bedroom house and contain optics, electronics, and software so sophisticated that their use often requires the technical and scientific expertise of specialists such as the ones in the Microscopy Center and Imaging core facilities.

Given the pace of technological change and the ever-increasing complexity of unsolved research problems, we believe that working with a team of experts is critical for the modern research enterprise. While scientists in the nineteenth and early twentieth centuries often worked alone, today's scientists almost never do. Promoting a team-based approach to science is one of the Institute's key institutional objectives. The importance of this objective is highlighted by the investment of a third of our scientific budget in the Core Facility teams and by our investment in programs designed to promote interaction and the formation of *ad hoc* teams.

I hope you will enjoy this issue's articles on chromosome dynamics, not only as examples of today's cutting-edge research but also as the continuation of a fascinating and illuminating line of inquiry that began over a hundred years ago.

Further reading:

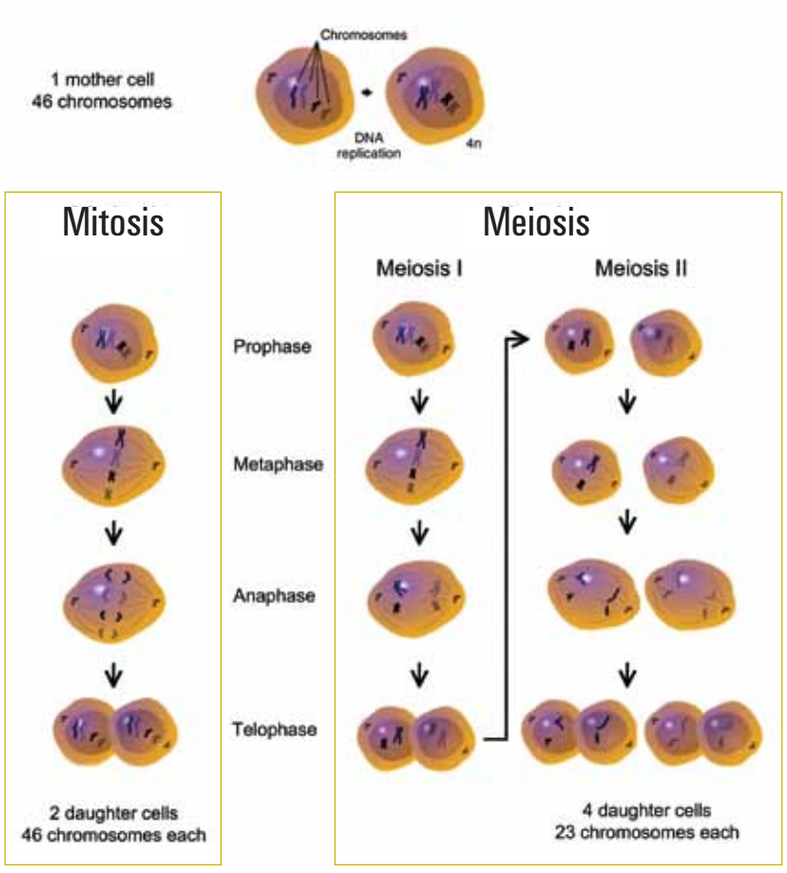
Garfield, Simon. *Mauve: How One Man Invented a Color That Changed the World.* New York: W.W. Norton & Company, 2002.
Lima-de-Faria, A. *One Hundred Years of Chromosome Research and What Remains to Be Learned.* Kluwer Academic, 2010.

By Stephanie Huang, Ph.D.

THE NOT-SO-SECRET LIF

An Introduction to Chromosome Dynamics During Cell Division

THE SIX BILLION BASE PAIRS OF DNA INSIDE A TYPICAL HUMAN CELL ARE FOLDED, TWISTED, AND ORGANIZED INTO 23 PAIRS OF CHROMOSOMES. TO PRESERVE THE INTEGRITY OF THIS GENETIC INFORMATION, THE CHROMOSOMES MUST REPLICATE AND EQUALLY SEGREGATE EACH TIME A CELL DIVIDES. THE CELLULAR COMPONENTS THAT WORK TO ENSURE ERROR-FREE CELL DIVISION ARE THE SUBJECTS OF STUDY IN THE FIELD OF CHROMOSOME DYNAMICS.



Adapted from images from the National Human Genome Research Institute, National Institutes of Health

This illustration shows a side-by-side comparison of the events in mitosis and meiosis. Normal human cells contain 23 pairs of chromosomes, or 46 chromosomes in total. Mitotic cell division produces two daughter cells with 46 chromosomes each. Meiotic cell division produces four daughter cells with 23 chromosomes each.

Chromosome dynamics in the simplest sense refers to the movements of chromosomes during cell division. Critical to these movements are the internal organization of the chromosomes, protein structures that are part of the chromosomes, protein structures that align and segregate the chromosomes during cell division, and protein signaling networks that coordinate the timing and location of all the various players.

Errors that result in the misplacement of a chromosome or part thereof can lead to a wide range of genetic diseases and cancers. Errors that occur during the production of the egg and sperm may result in sterility or genetic disorders such as Down syndrome. Errors that occur during the division of cells in the body can produce cells with misregulated growth and the potential to become cancerous.

Basics of Cell Division

As an introduction to the articles in this issue of the *Stowers Report*, we outline below some of the important steps that occur during cell division and contribute to chromosome dynamics.

Most of the cells in our body undergo a type of cell division called *mitosis*. Mitotic cell division produces two daughter cells that contain the same number of chromosomes as the starting mother cell – in humans, 23 pairs of chromosomes or 46 chromosomes in total. In preparation for mitosis, each chromosome in the cell replicates, or makes a copy of itself. The two copies of each chromosome, called sister chromatids, remain joined at their centers at a junction called the centromere. Mitosis then proceeds through a series of four steps or phases – prophase, metaphase, anaphase, and telophase – as illustrated in the image on the left.

In nondividing cells, chromosomes are loosely bundled. At the start of mitosis, in prophase, chromosomes fold and twist tightly to form highly condensed structures. At the end of prophase, the nuclear membrane, which normally encloses the chromosomes, dissolves. This allows protein cables

E OF CHROMOSOMES

called microtubules to penetrate into the nuclear space and form a football-shaped structure called the spindle. The spindle microtubules attach to the centromeres of each chromosome. In metaphase, these microtubules push and prod the chromosomes into alignment in the center of the cell. In anaphase, having aligned the chromosomes, the microtubules now pull the sister chromatids apart and pull one chromatid of each pair to the opposite ends of the cell. In telophase, a new nuclear membrane forms around the two equal masses of chromosomes. With mitosis complete, the cell divides, forming two daughter cells.

While most of our cells undergo mitotic cell division, the reproductive cells, the egg and sperm, are formed by way of meiotic cell division. In *meiosis*, the events are similar to that of mitosis, but the mother cell divides twice, producing four daughter cells, as illustrated in the image on page 2. Each of the four daughter cells contains only one copy of each chromosome, or 23 chromosomes in total. In sexual reproduction, meiosis is complemented by fertilization, which fuses an egg cell and sperm cell, creating a cell with two copies of each chromosome, or 46 chromosomes in total.

At the Institute

At the Stowers Institute, a number of research teams focus their work on understanding how gene and protein components work together to regulate the movements of chromosomes during cell division. With the aim of elucidating the basic processes that underlie chromosome dynamics, these investigators hope to shed light on what can go wrong, which components might be involved, and, ultimately, how to fix those errors and prevent disease.

Peter Baumann, Ph.D., Associate Investigator, and his team largely focus their work on understanding how the enzyme telomerase maintains the ends of chromosomes, or telomeres. Defects in telomere maintenance can contribute to human diseases, including cancer and premature aging. A few years ago, the Baumann Lab became interested in the phenomenon of parthenogenesis, a form of asexual reproduction found in some animal species in which females reproduce without fertilization by a male. Early last year, in collaboration with other scientists at the Institute, the team described a unique chromosome pairing mechanism in parthenogenetic lizards that enables female lizards to maintain the existing genetic variation present in their genome. This story was featured in the Spring 2010 issue of the *Stowers Report*.

Jennifer Gerton, Ph.D., Associate Investigator, and her team study processes that are critical for the fidelity of chromosome distribution. One of the processes they study is chromosome cohesion, the process by which sister chromatids are bound together through the end of metaphase. Another process they study is the regulation of the centromere, the chromosomal attachment point for sister chromatids and spindle microtubules. Recently, in collaboration with other scientists at the Institute, the Gerton Lab revealed protein players that are critical for establishing and maintaining exactly one centromere per chromosome. This story is on page 7.

R. Scott Hawley, Ph.D., Investigator, and his team seek to understand the regulation of chromosome behavior during meiosis. A distinguishing event in meiosis is the occurrence of pairing and recombination between a pair of homologous chromosomes (one inherited from the mother and the other inherited from the father). Recombination, or chromosome crossing over, serves the vital function of linking homologous chromosomes together and thus ensuring their segregation. In addition to studying how meiotic chromosomes pair and recombine, the Hawley Lab is also interested in protein signaling networks that control the progression through meiosis. Last summer, they published a report on a protein called calcineurin and its regulator Sra in which they show both proteins are essential for the last stage of chromosome movement towards the two opposite spindle poles. This story is on page 10.

Sue Jaspersen, Ph.D., Assistant Investigator, and her team focus their work on the study of proteins residing in the inner nuclear membrane and how those proteins regulate the non-random distribution of chromosomes within the nucleus. Mutations in these proteins can lead to a number of inherited diseases and cancers. One of these proteins, Mps3, is a critical component of the yeast spindle pole body, which duplicates early in cell division to form the poles of the spindle. Along with collaborators, the Jaspersen Lab recently revealed an unexpected relationship between Mps3, nuclear pore complexes, and the lipids of the nuclear membrane. This story is on page 13.

Rong Li, Ph.D., Investigator, studies the mechanisms that underlie cell shape and organization. Among various topics such as cell polarity, asymmetric cell division, and polycystic kidney disease, the Rong Li Lab also studies adaptive evolution and the biological machinery that enables cells to adapt to changes in their internal and external environments. Their work led them to study aneuploidy, a condition in which cells have an abnormal number of chromosomes. While aneuploidy is usually associated with genetic defects and disease, the team had previously found that aneuploidy can enable rapid adaptive changes. They recently published results that show that aneuploidy can indeed be beneficial under certain cellular circumstances. This story is on page 4.

By Elise Lamar, Ph.D.

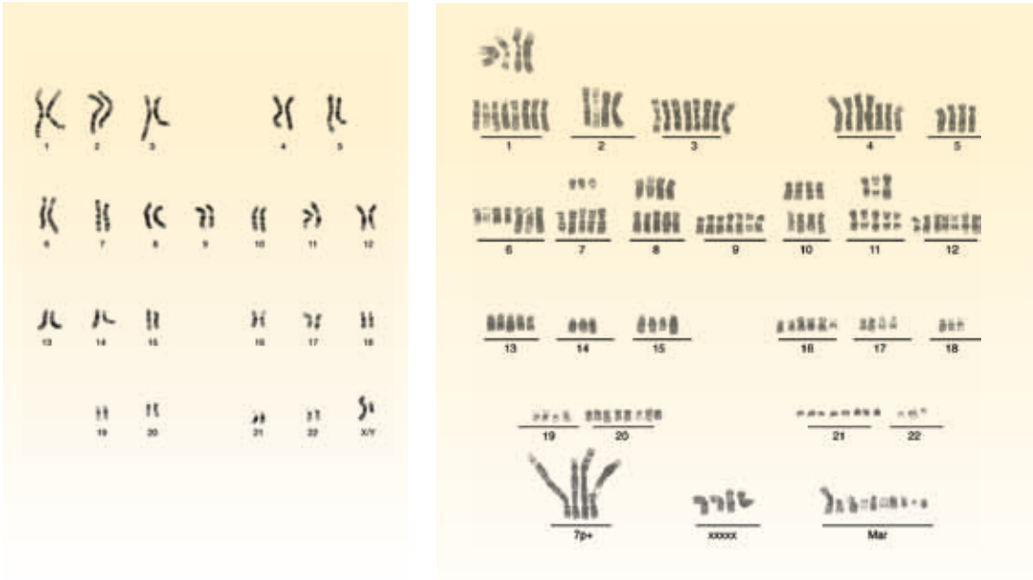
EVOLUTION AND CANCER

THE AXIOM "SURVIVAL OF THE FITTEST" UNDERLIES EVOLUTIONARY THEORY. ORGANISMS GENETICALLY EQUIPPED TO SURVIVE ENVIRONMENTAL HARDSHIP ARE MORE LIKELY TO REPRODUCE THAN LESS FIT INDIVIDUALS. HOWEVER, WITHIN AN ORGANISM, CELLS SEEMINGLY "UNFIT" BY HUMAN STANDARDS - SUCH AS CANCER CELLS - CAN OUTSTRIP HEALTHY CELLS IN CONFRONTING HOSTILE OR RESTRICTIVE ENVIRONMENTS. DEFINING THE GENETIC BASIS OF THAT INSIDIOUS "FITNESS" COULD LEAD TO MORE EFFECTIVE WAYS TO ERADICATE TUMORS.

One anomaly displayed by cancer cells, the possession of extra copies of one or more chromosomes, is of intense interest to Rong Li, Ph.D., Investigator. Her team has recently used yeast cells to show that chromosome number abnormalities, referred to as aneuploidy, occasionally give cells a survival edge in adversity, potentially explaining cancer cells' remarkable endurance.

Experimenting with Evolution

The Rong Li Lab evaluates evolutionary fitness experimentally, which may seem improbable given evolution's glacial pace. Nonetheless, evolution of cells can occur on a tractable time scale, and recent advances in techniques to rapidly assess DNA copy number and sequence in entire genomes now make "experimental evolution" analysis much more feasible. These studies often employ fast-replicating simple organisms as model systems to define molecular processes necessary for adaptation to a hostile environment.



Aneuploidy can be determined by staining and examining the chromosomes of a cell. Sorting the images of these chromosomes into their respective pairs makes it easier to visualize cases of aneuploidy. On the left is a typical set of chromosomes for a human male —there are exactly two copies of each chromosome (except for the sex chromosomes). On the right is a set of chromosomes from a leiomyosarcoma cell, an example of a highly aneuploid tumor cell. Leiomyosarcoma is a cancer of smooth muscle. There are at least three copies of each chromosome, as well as chromosome rearrangements formed by broken-off pieces of chromosomes. Chromosomes labeled 7p+ were not present in all of the tumor cells, and chromosomes marked "Mar" are chromosomes of uncertain origin.

Left: National Human Genome Research Institute, National Institutes of Health
Right: Adapted from van de Rijn et al. Annu Rev Pathol. *2006. 1:435-66. Copyright 2005 Annual Reviews, Inc. Reprinted with permission.*

Defining the Molecular Basis of "Fitness"

For their recent study, the team used the single-cell budding yeast *Saccharomyces cerevisiae* – a favorite of bakers and brewers. Dr. Li describes herself as a basic cell biologist, but ideas about cancer permeate her thinking. "Our yeast work does not directly address cancer," she says. "But the current debate about what kinds of genetic changes cause cancer is always in the back of our minds."

That debate began to assume a prominent position in the team's research when they discovered that aneuploidy had unanticipated consequences for yeast. Aneuploidy has had a well-deserved bad reputation: almost all cancer cells show aberrant numbers of some chromosomes, and a current debate in cancer research is whether aneuploidy is a consequence or a cause of cancer. Aneuploidy is also associated with birth defects: most human embryos carrying aberrant chromosome numbers die *in utero*. Notable exceptions are Down syndrome, marked by three copies of chromosome 21, and sex chromosome anomalies in which individuals harbor aberrant numbers of X or Y chromosomes.

Aneuploidy: Jekyll or Hyde?

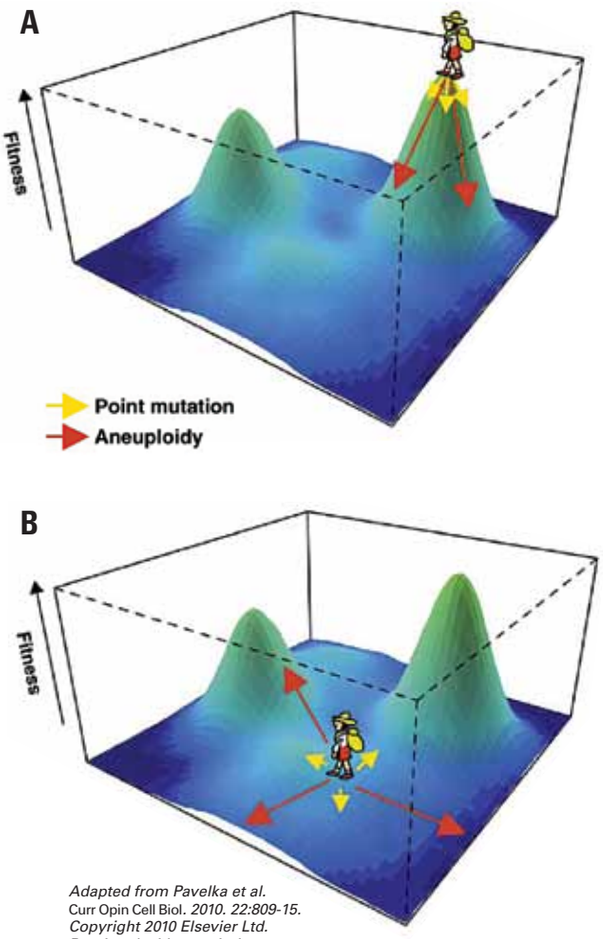
Despite aneuploidy's disastrous consequences for whole organisms, the Rong Li Lab recently began asking whether aneuploidy might be beneficial from a *cellular* point of view. Those ideas emerged following their 2008 study published in *Cell* in which the lab discovered that yeast harboring extra chromosomes survived the loss of a gene normally required for reproduction.

Saccharomyces reproduces by budding a daughter from the mother yeast cell. In the *Cell* study, the group mutated a gene required for budding, killing most mutants. But a few mutants limped along through hundreds of cell divisions and gradually "evolved" strategies to circumvent loss of the critical gene. In the end, some mutants divided as happily as normal cells. Significantly, all survivors showed abnormal numbers of a subset of chromosomes, suggesting that induction of aneuploidy – detrimental in normal circumstances – can enable mortally wounded cells to thrive.

The presence of aneuploidy in all survivors made Dr. Li reconsider the evolutionary advantage of acquiring big chunks of chromosomal material: "If an organism is exposed to acute stress conditions, they might need to respond rapidly and invent new functions, which would be particularly challenging if cells had never encountered those conditions," she says. "Aneuploidy might be a very effective way for an organism to make rapid adaptations to acute stress."

Context is All

That hypothesis formed the basis of the current study, which was published online in *Nature* on October 20, 2010. The team that drove the study was led by a trio of first authors, Norman Pavelka, Ph.D., Postdoctoral Research Associate, Giulia Rancati, Ph.D., Postdoctoral Research Associate, and Jin Zhu, Research Technician I and Graduate Student. The team first engineered a population of 38 yeast "misfits," each possessing an aberrant number of *Saccharomyces'* 16 chromosomes. One strain had extra chromosomes 2 and 12, another 9 and 16, and so forth, while others had multiple duplicated chromosomes. In collaboration with the Proteomics, Molecular Biology, and Bioinformatics core facilities, the team subjected engineered yeast to an onslaught



Adapted from Pavelka et al.
Curr Opin Cell Biol. *2010. 22:809-15.*
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Figure A is a scenario in which a cell (represented by the hiker) is fit and healthy. Small genetic changes like point mutations may allow the cell to remain fit (atop or near the top of the fitness peak), but large genetic changes like aneuploidy will typically make the cell unfit (push it down the peak). Figure B is a scenario in which the cell is unfit because of genetic or environmental changes. Point mutations are unlikely to make a significant difference in the fitness of the cell, but aneuploidy enables the cell to make large genetic changes, in some cases bringing it closer to a fitness peak and allowing it to survive adverse conditions.

of laboratory stresses and evaluated their "fitness" based on their ability to reproduce.

Overall, the dogma regarding the perils of aneuploidy was confirmed: aneuploid yeast grew poorly, and were thus "unfit," in conditions favorable to normal yeast. But when confronted by environmental insult, such as low temperature, starvation, acid pH, or toxic drugs, some aneuploid strains showed a growth advantage over yeast with normal chromosome numbers, suggesting they had hidden reserves.

Survival of the Well-Stocked

Why apparently "unfit" yeast mutants thrive in an unfavorable milieu is complex and likely differs from mutant to mutant. Yeast chromosomes contain hundreds of genes, and the team also showed that possession of an extra chromosome allowed mutants to simply make a larger amount of protein products encoded on duplicated chromosomes. Mutant survivors likely exploit that overabundance to survive.

A simple example is the *ATR1* gene, carried on yeast chromosome 13. *ATR1* provides the genetic blueprint for a protein used to flush a specific toxin out of cells. When the investigators exposed both normal and aneuploid yeast to that toxin, mutants carrying an extra copy of chromosome 13 survived better than did normal cells or most other aneuploid strains, suggesting they could construct a greater number of pipelines to rid cells of the toxin than could yeast lacking an extra chromosome 13.

These findings suggest the following scenario. In the "real world," aneuploid cells emerge infrequently and by accident as cells divide. Many probably die. The ones that survive harbor a surfeit of potentially useful protein tools. In good times, aneuploid cells do not use those tools and, in fact, might find them burdensome given their sluggish growth. But when cells face environmental catastrophe, extraneous proteins constitute a survival kit to counteract a range of unforeseen hazards. In short, in the "right" context, aneuploidy confers a selective advantage that "normal" cells lack.

A New Approach to Cancer: Hitting a Moving Target

Like aneuploid yeast survivors, cancer cells are adept at thwarting attempts to eradicate them. Not only do they hide themselves from the host's immune system, but over time most cancers become resistant to chemotherapies or radiation. "Most existing chemotherapy targets specific pathways, like growth factors or the cell cycle machinery," says Dr. Li. "We hit one growth factor receptor and may kill a cell, but then a new tumor emerges – we are always behind, chasing this disease, because from an evolutionary perspective, a tumor cell, possibly through becoming aneuploid, creates new ways to survive through genomic change."

As an alternative, Dr. Li hopes her investigations will encourage cancer researchers to consider means to tinker with the global, genomic mechanisms that make tumor cells so versatile. "Cancer is a moving target," she says, suggesting that what is needed is a way to block cancer cells' remarkable ability to dodge bullets. "Effective cancer therapies must not only rapidly kill proliferating tumor populations, but also break the vicious cycle of tumorigenesis by preventing increases in aneuploidy and other adaptive mutations."

PAPER: Aneuploidy Confers Quantitative Proteome Changes and Phenotypic Variation in Budding Yeast

JOURNAL: *Nature*

ISSUE: November 11, 2010 (published online October 20, 2010)

AUTHORS*: **Norman Pavelka, Ph.D.**, Postdoctoral Research Associate; **Giulia Rancati, Ph.D.**, Postdoctoral Research Associate; **Jin Zhu**, Research Technician I; **Dan Bradford**, Research Specialist I; **Anita Saraf, Ph.D.**, Senior Proteomics Scientist; **Laurence Florens, Ph.D**, Head of Proteomics; **Brian Sanderson**, formerly Senior Laboratory Manager; **Gaye Hattem**, Programmer Analyst II; **Rong Li, Ph.D.**, Investigator

**Authors' primary appointments are with the Stowers Institute for Medical Research.*

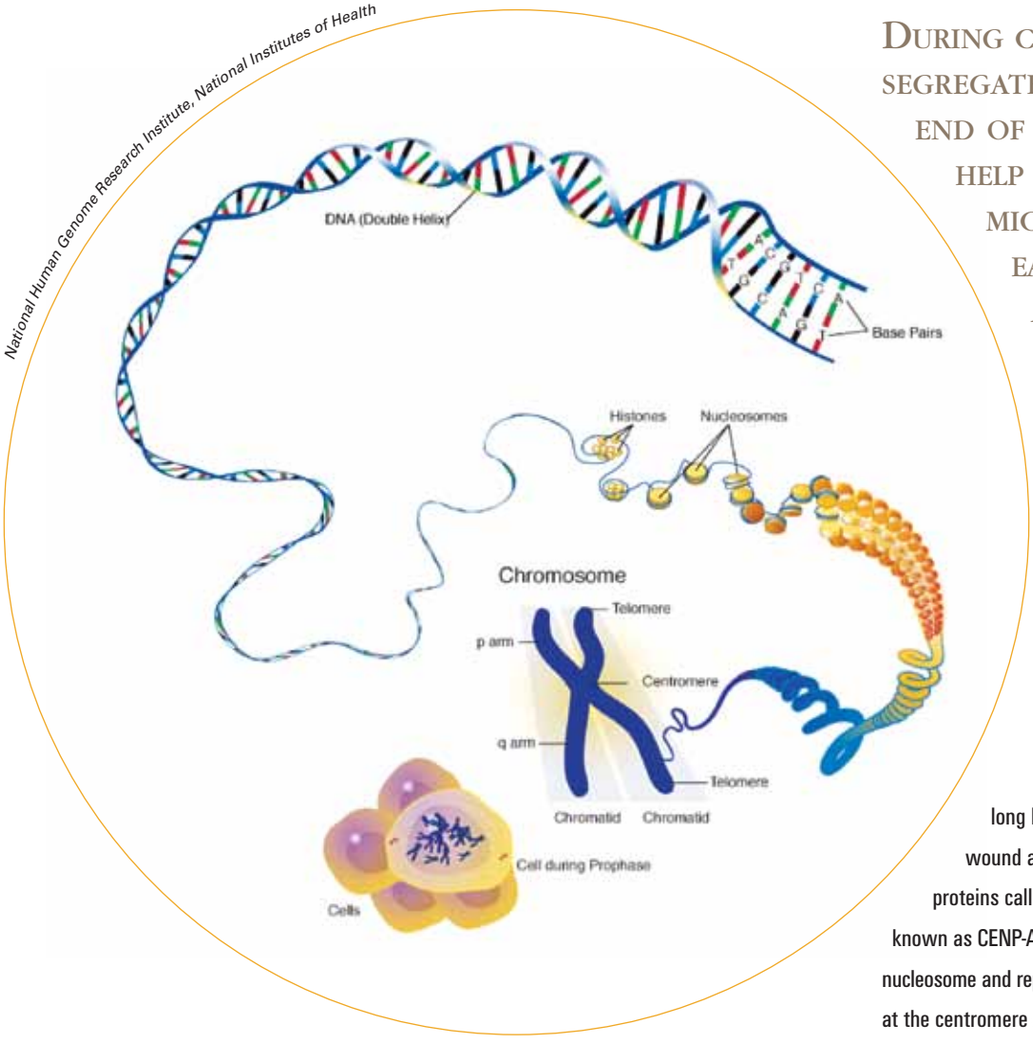
Rong Li, Ph.D., Investigator, is also a Professor in the Department of Molecular and Integrative Physiology at the University of Kansas Medical Center. Learn more about her work at http://www.stowers.org/labs/RongLiLab.asp.

By Stephanie Huang, Ph.D.

A CELLULAR MURDER MYSTERY

Identification of Psh1 as the Ubiquitin Ligase for Cse4

National Human Genome Research Institute, National Institutes of Health

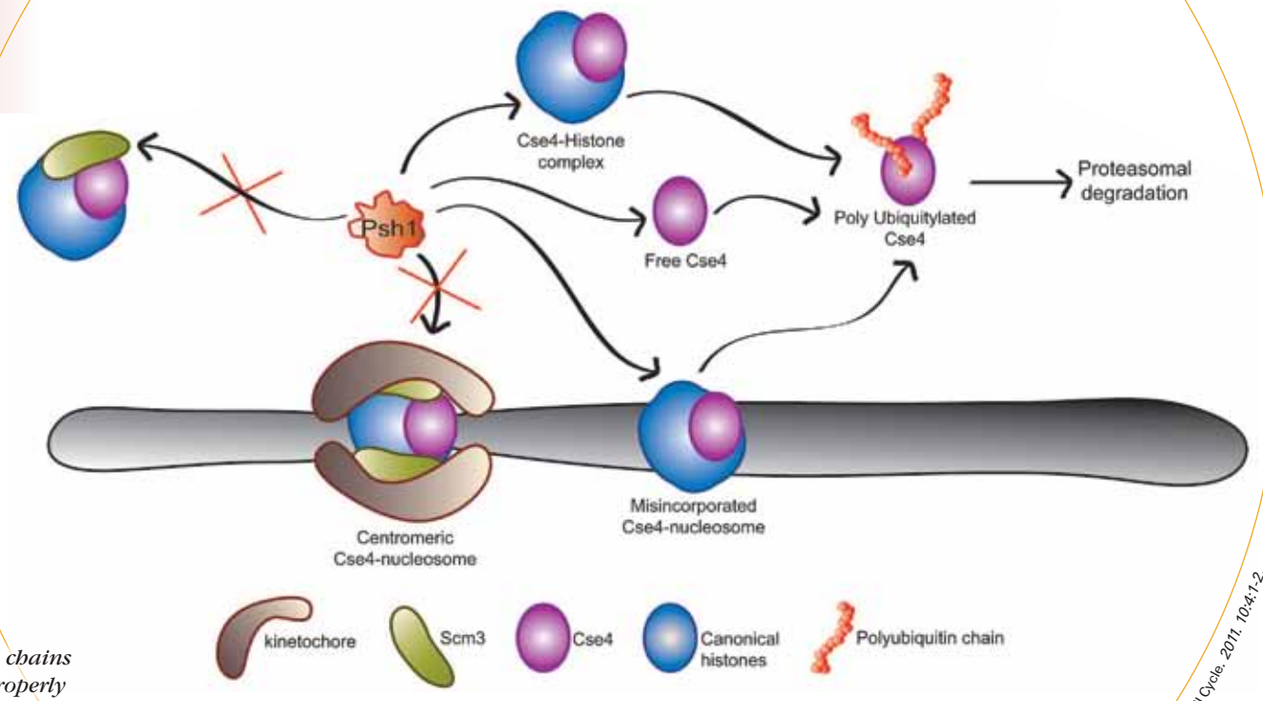


DNA strands are wrapped around histone-containing nucleosomes. The DNA-nucleosome structure is then further coiled and folded into chromosomes. At yeast centromeres, Cse4 replaces one of the histones at exactly one nucleosome. This small change is sufficient to mark the centromere.

During cell division, chromosomes are segregated and distributed to either end of the dividing cell with the help of molecular cables called microtubules. In order to move each chromosome, microtubules attach to an anchoring site on the chromosome called the centromere. Proper regulation of the centromere is thus extremely critical to cell division — a misplaced centromere can lead to uneven distribution of DNA material and severe consequences for the cells.

What makes a centromere a centromere? In order to pack long DNA strands into a chromosome, the strands are first wound around nucleosomes, which are clusters of DNA-packaging proteins called histones. At the centromere, a histone-like protein, known as CENP-A in humans and Cse4 in budding yeast, sneaks into the nucleosome and replaces one of the histones. In yeast, exactly one nucleosome at the centromere contains Cse4. This small change is sufficient to mark the centromere and distinguish it from surrounding DNA material.

How does Cse4 find the single nucleosome at each centromere and not get lost among the 60,000 or so other nucleosomes? Led by Geetha Hewawasam, Ph.D.,



Psh1 adds ubiquitin chains to Cse4 that is not properly bound to the centromere, targeting those Cse4 proteins for ubiquitin-mediated proteasome degradation. Scm3 protects Cse4 from Psh1.

Hewawasam et al. Cell Cycle. 2011. 10:4:1-2.

Research Specialist I in the Gerton Lab, and Jennifer Gerton, Ph.D., Associate Investigator, a team at the Institute has recently identified two proteins that interact with Cse4 and help to ensure that it incorporates only at the centromere and not elsewhere on the chromosome. They reported their findings in the November 12, 2010 issue of *Molecular Cell.*

Marked for Elimination

Previous work in the field had suggested that ubiquitin-mediated degradation, a process of breaking down proteins, might be involved in targeting Cse4 to the centromere. Proteins are constantly synthesized and broken down during the course of a cell's life. There are various reasons that a particular protein is broken down, or degraded – a protein may have been synthesized for a specific purpose and is no longer needed afterwards, or it might be defective and cannot function properly.

A protein marked for degradation becomes literally marked – with long chains of a small protein called ubiquitin. These ubiquitin chains lead the doomed protein to the proteosome, a large protein machine that breaks up the doomed protein into its component amino acids and recycles those amino acids to create new proteins.

A key player in protein degradation is the ubiquitin ligase. Ligase comes from a Latin verb meaning "to glue together" – the ubiquitin ligase attaches ubiquitin chains to the doomed protein. For each protein that is tagged with ubiquitin, there must be a specific ligase that does the tagging.

Murder Mystery

Ubiquitin-mediated degradation is thought to regulate Cse4 by degrading any Cse4 proteins that are not found at the centromere. Cse4 bound to the centromeres appear to be protected from degradation, while other Cse4 proteins are degraded.

In a sense, this is a cellular murder mystery. We know the targets (non-centromeric Cse4 proteins) and we know the assassin (the proteosome). We are missing two important players. The first is the bodyguard – who is protecting centromere-bound Cse4? The second is the messenger – who is tagging the non-centromeric Cse4 and leading them to the proteosome for degradation?

The Messenger and the Bodyguard

In collaboration with the Proteomics Center, the team identified an uncharacterized protein called Psh1 that interacts with Cse4. Then, using biochemical experiments, the team showed that Psh1 functions as a ubiquitin ligase for Cse4. In other words, Psh1 is the messenger in this murder mystery – it attaches the ubiquitin chains to Cse4 in order to lead Cse4 to the proteosome for degradation.

The team also revealed that Scm3, a protein that recruits Cse4 to the centromere, might be the bodyguard. In the test tube, having Scm3 around prevents Psh1 from adding ubiquitin chains onto Cse4. The team's yeast genetic experiments confirm that Scm3 likely protects Cse4 from Psh1.

Further genetic experiments completed the picture. Scm3 appears to protect from degradation any Cse4 proteins that have found the centromere, and Psh1 degrades any Cse4 that is not at the centromere. To test this scenario, the team constructed a yeast strain in which they deleted the *PSH1* gene and flooded the cell with high levels of Cse4. As a result, there was nearly four times the normal amount of Cse4 at the centromeres, and Cse4 was found in regions of DNA where it is not normally found – thus supporting the idea that having Psh1 around prevents Cse4 from landing on the wrong, non-centromeric sites.

With Psh1 identified, the team hopes to move forward in answering other important questions about this system. Although the study revealed that Scm3 plays a role in protecting Cse4, Dr. Gerton thinks the key will be to understand the structure of the centromeric nucleosome. It is unclear whether the centromeric nucleosome resembles the canonical nucleosome that is present everywhere else in the chromosome or whether it differs entirely.

"There is a debate in the field about what the centromeric nucleosome looks like," explains Dr. Gerton. "Is it octameric like the canonical histone? Is it hexameric? Is the DNA wrapped in the opposite direction? The structure is controversial, but may very well reveal how [Cse4] is regulated or protected."

Consistent with the centromere's role in chromosome segregation and thus genomic stability, excess CENP-A is associated with human colorectal cancer. Extending the team's findings from yeast to human will help in understanding how centromeric sequences are regulated in our own cells.

PAPER: Psh1 Is an E3 Ubiquitin Ligase that Targets the Centromeric Histone Variant Cse4

JOURNAL: *Molecular Cell*

ISSUE: November 12, 2010

AUTHORS*: **Geetha Hewawasam, Ph.D.**, Research Specialist I; **Manjunatha Shivaraju**, Predoctoral Researcher; **Mark Mattingly**, Research Technician II; **Swaminathan Venkatesh, Ph.D.**, Postdoctoral Research Associate; **Skylar Martin-Brown**, Research Technician III; **Laurence Florens, Ph.D.**, Head of Proteomics; **Jerry Workman, Ph.D.**, Investigator; **Jennifer Gerton, Ph.D.**, Associate Investigator

**Authors' primary appointments are with the Stowers Institute for Medical Research.*

Jennifer Gerton, Ph.D., Associate Investigator, is also an Associate Professor in the Department of Biochemistry and Molecular Biology at the University of Kansas School of Medicine. Learn more about her work at http://www.stowers.org/labs/GertonLab.asp.

Jerry Workman, Ph.D., Investigator, joined the Stowers Institute in 2003 from The Pennsylvania State University where he was an Associate Investigator of the Howard Hughes Medical Institute. Learn more about his work at http://www.stowers.org/labs/WorkmanLab.asp.

By Stephanie Huang, Ph.D.

LIVING PROOF
THE ESSENTIAL ROLE OF

THE DEVELOPMENT OF THE EGG CELL IS CAREFULLY REGULATED TO ENSURE THAT EACH CELL RECEIVES THE CORRECT NUMBER OF CHROMOSOMES. ERRORS CAN LEAD TO AN ABNORMAL NUMBER OF CHROMOSOMES, OR ANEUPLOIDY, RESULTING IN THE DEATH OR INVIABILITY OF THE EGG OR SERIOUS GENETIC DEFECTS IN THE DEVELOPED ORGANISM.

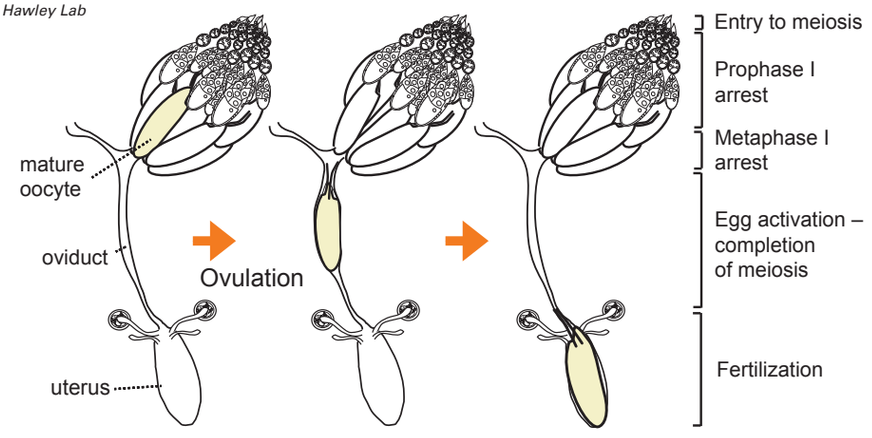
Egg cells are formed by the meiotic cell division of immature cells called oocytes. Meiosis is a form of cell division in which the steps closely resemble those in mitosis, but the starting cell divides twice. Metaphase is the point at which the chromosomes are aligned in a single row in the middle of the cell. Anaphase is the point at which the replicated chromosomes split apart, and protein cables known as microtubules pull one chromosome of each replicated pair to opposite ends of the cell. Because the cell divides twice in meiosis, there are two metaphases and two anaphases (e.g., metaphase I and metaphase II).

In frogs and mice, oocytes are arrested at metaphase II, just before the final cell division that produces the mature egg cell. Only upon fertilization are the oocytes released from cell cycle arrest. Fertilization results in, among other things, a rise in the concentration of calcium within the cell. This spike in calcium serves as a signal to various proteins and sets off a chain of events that ultimately culminate in the successful completion of meiosis.

Egg development proceeds through a slightly different path in the fruit fly, *Drosophila melanogaster. Drosophila* oocytes arrest earlier in comparison with other oocytes – they arrest at metaphase I – and cell cycle release occurs upon ovulation, not fertilization. It was not obvious that calcium-signaling would play a critical role in *Drosophila* female meiosis.

Drosophila has historically served as an ideal model organism in which to study cell division and the cell cycle, because of its quick division times, well-developed genetic tools, and detailed cytology. Using *Drosophila* oocytes, Satomi Takeo, Ph.D., Postdoctoral Research Fellow in the Hawley Lab, and R. Scott Hawley, Ph.D., Investigator, have provided direct evidence in support of the essential role played by a calcium-activated protein called calcineurin during *Drosophila* female meiosis. Their work was published online in *Developmental Biology* on June 16, 2010.

Hawley Lab



Schematic of reproductive system of female Drosophila, *showing progression of the oocyte and associated meiotic stages.*

The Question

Back in 2006, before she joined the Institute, Dr. Takeo and colleagues published a study in *Current Biology* hinting at the critical role of calcineurin in *Drosophila* female meiosis. This was the first evidence in any organism that calcineurin might be critical for female meiosis. In the year after their study, two other studies were published showing that calcineurin is required for cell cycle release in oocytes from the frog, *Xenopus laevis*.

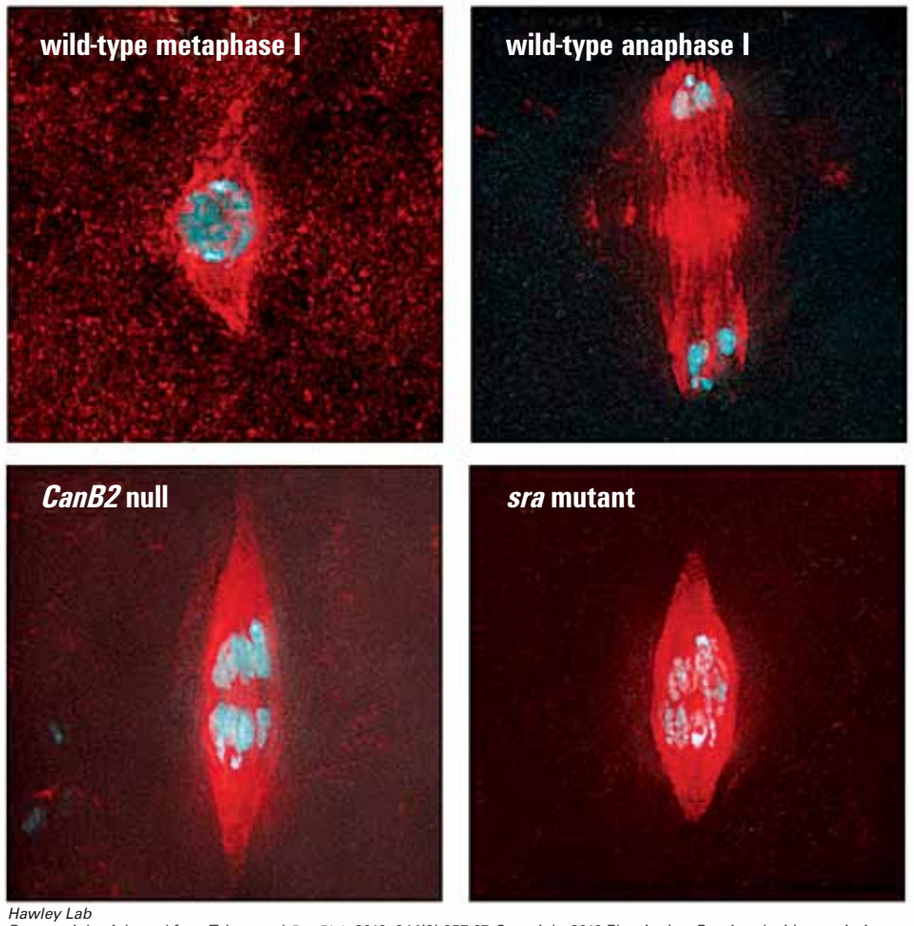
CALCINEURIN IN *DROSOPHILA* FEMALE MEIOSIS

Although these studies suggested that calcium-signaling and calcineurin might be important for *Drosophila* oocyte development, the field lacked direct evidence supporting this fact. "People expected that calcium-signaling was going to play a critical role in *Drosophila* meiosis," remarks Dr. Hawley. "But there's a difference between expectation and proof. Satomi's [current] work gave us the proof and the players in the pathway."

The Players

Calcineurin is a calcium-dependent phosphatase. In the presence of high calcium concentrations – such as during fertilization of oocytes – calcineurin is activated. Calcineurin removes a chemical modification called phosphorylation from other proteins, thus modifying the functions of these proteins. Calcineurin is composed of two subunits – subunit A (CnA) provides the catalytic function (the dephosphorylating action), and subunit B (CnB) is a regulatory unit.

In addition to a regulatory subunit, calcineurin is also regulated by the RCAN (regulators of calcineurin) family of proteins. The *Drosophila* gene *sra* encodes an RCAN family member. Dr. Takeo's work in 2006 showed that *sra* is essential for meiotic progression in oocytes, thus hinting that calcineurin may also be critical for female meiosis.



Hawley Lab
Bottom right: Adapted from Takeo et al. Dev Biol. 2010. 344(2):957-67. Copyright 2010 Elsevier Inc. Reprinted with permission.

In these images, DNA is stained in blue, and microtubules are stained in red. Wild-type non-mutant oocytes show expected distribution of chromosomes at metaphase (center of the cell) and anaphase (segregated to opposite ends of the cell). In eggs deficient for calcineurin or Sra function, the laid eggs arrest with stalled chromosomes that have moved only partway towards their respective poles.

The Proof

Taking advantage of the genetic tractability of *Drosophila*, Dr. Takeo proceeded to provide the direct proof that calcineurin is indeed essential for female meiosis in *Drosophila*. She obtained a null, or non-functional, allele of a gene encoding one of the CnB subunits, *CanB2*. Oocytes with the *CanB2* null mutant displayed an interesting defect. The laid eggs failed to hatch, and microscopy analysis revealed that the chromosomes had stalled – in other words, the chromosomes had separated and started to migrate

towards their opposite poles, but appeared to stop halfway there.

Since *CanB2* is the only CnB subunit gene expressed in the ovary, the team was able to conclude that these mutant oocytes contained no functional calcineurin, and thus calcineurin was essential for female meiosis in *Drosophila*.

The Details

Dr. Takeo's previous work had demonstrated that *sra* mutants had the same curious meiotic defect. Knowing that *sra* encodes a regulator of calcineurin, this is not terribly surprising. However, the fact that mutations in *sra* and *CanB2* produce the same defect confirmed that the two proteins encoded by these genes are working with each other during *Drosophila* oocyte meiosis. With further genetic experiments, Dr. Takeo showed that *sra* positively regulates calcineurin.

With a series of other experiments, Dr. Takeo began to flesh out the mechanisms of how the Sra protein itself might be regulated. Previous studies have shown that RCAN proteins are regulated by phosphorylation on two specific locations on the protein. As discussed earlier, phosphorylation is a type of chemical modification that can activate or inhibit the function of the affected protein.

Mutating the sites of phosphorylation — or deleting them altogether — is a common way for researchers to prevent phosphorylation of a given protein and determine whether the chemical modification does indeed affect function. Using these techniques, Dr. Takeo showed that phosphorylation at a specific site on Sra is essential for Sra function and calcineurin activation in *Drosophila* female meiosis.

The Future

Despite the differences in *Xenopus* and *Drosophila* oocyte development, it seems that the players are very similar. "The pathway may be conserved even if the trigger for meiotic release is different," observes Dr. Takeo.

One unanswered question of interest is how Sra and calcineurin are affecting chromosome movement and why the chromosomes appear to stall partway towards the poles. Dr. Hawley and Dr. Takeo point out that in order to answer this question, they first need to find more pieces of the puzzle.

For one thing, the substrates of calcineurin during *Drosophila* meiosis are not yet known. Now that the role of calcineurin has been established, the next step will be to identify which proteins are dephosphorylated and regulated by calcineurin.

Even more intriguing, understanding the regulation of Sra and other RCAN family members may provide insight into the development of Down syndrome and other diseases. The human RCAN gene, *RCAN1*, is located on chromosome 21, and, in fact, was originally named *DSCR1* for Down syndrome critical region 1. Besides Down syndrome, *RCAN1* also appears to be involved with heart failure, growth of blood vessels in tumors, and Alzheimer's disease. Using a model system like *Drosophila* may facilitate work to understand the role of RCAN members in meiosis and other cellular processes and what can go wrong.

For now, the team is happy to have provided a solid contribution to the field. "This has been an incredibly well-received article," remarks Dr. Hawley. "This work is pulling together a lot of important threads and has given us a window to look into what calcium is doing in the oocyte."

PAPER: Calcineurin and its Regulation by Sra/RCAN is Required for Completion of Meiosis in *Drosophila*

JOURNAL: *Developmental Biology*

ISSUE: August 15, 2010 (published online June 16, 2010)

AUTHORS*: **Satomi Takeo, Ph.D.**, Postdoctoral Research Fellow; **R. Scott Hawley, Ph.D.**, Investigator; **Toshiro Aigaki, Ph.D.**, Tokyo Metropolitan University

**Authors' primary appointments are with the Stowers Institute for Medical Research unless otherwise noted.*

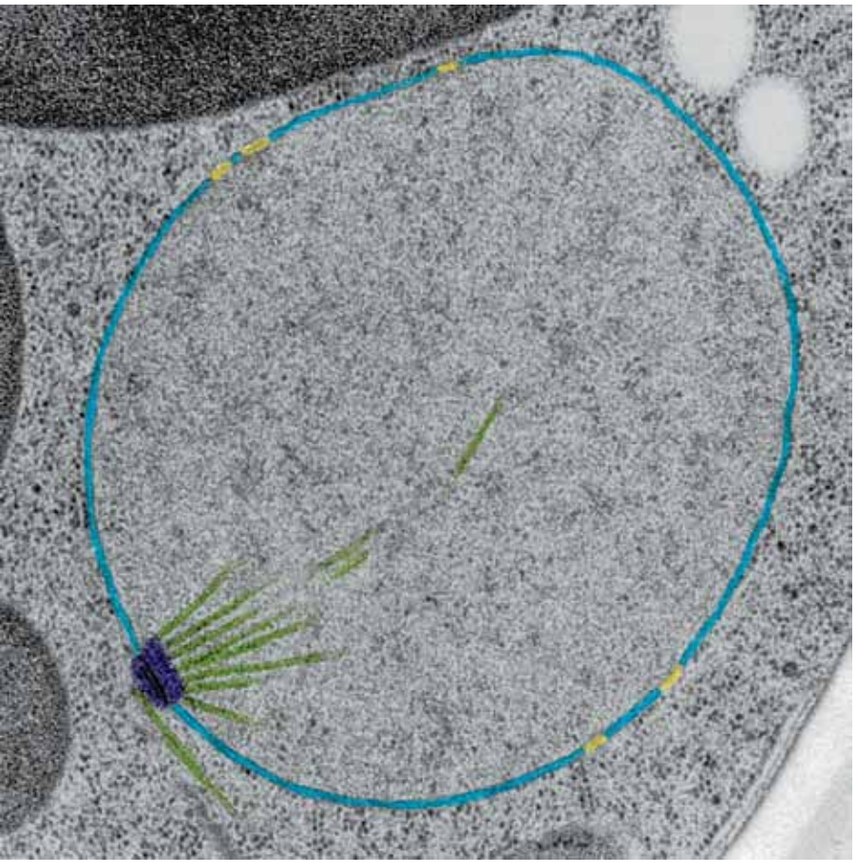
R. Scott Hawley, Ph.D., Investigator, is also Professor of Molecular and Integrative Physiology in the School of Medicine at the University of Kansas Medical Center; Adjunct Professor of Biological Sciences at the University of Missouri Kansas City; and Adjunct Professor of Undergraduate Program in Biology at the University of Kansas. Learn more about his work at http://www.stowers.org/labs/HawleyLab.asp.

By Stephanie Huang, Ph.D.

NOT SO PASSIVE AFTER ALL

Changes in the Nuclear Membrane Affect Spindle Pole Body Duplication

A double layer of fatty molecules called lipids encases each of our cells and many of the organelles within our cells. Classical cell biology teaches us that these membranes form important barriers between the cell and the outside environment and between different compartments within the cell.



Jaspersen Lab

An electron micrograph of the yeast nucleus shows nuclear pore complexes (yellow) and a spindle pole body (purple) embedded in the nuclear membrane (aqua). During mitosis, the spindle pole body will duplicate within the nuclear membrane and form the poles from which the spindle microtubules will emanate (green).

The compartment that holds the chromosomes is called the nucleus and is separated from the rest of the cell by the nuclear membrane. The nuclear membrane isolates our DNA in a specialized compartment and carefully regulates the proteins that are allowed to enter and exit the nucleus by way of specialized pores in the membrane.

These observations might suggest that membranes are passive inert structures. A recent study by a team that includes Jennifer Friederichs, Laboratory Assistant in the Jaspersen Lab, and Sue Jaspersen, Ph.D., Assistant Investigator, suggests otherwise and lends support to the idea that membranes are actively affecting the activity of the proteins embedded within them.

Mps3 and the Spindle Pole Body

At the start of mitosis in animal cells, an organelle called the centrosome duplicates, and the two new centrosomes move to opposite ends of the cell. From here, the centrosomes anchor protein cables called microtubules, which radiate from the centrosomes and reach inwards into the cell to attach to the replicated chromosomes. This forms the so-called mitotic spindle, a football-shaped structure that plays a critical role in aligning and segregating the replicated chromosomes. Towards the end of mitosis, the spindle microtubules pull apart the replicated chromosomes, equally distributing the chromosomes between the two forming daughter cells.

In most animal cells, the nuclear membrane dissolves to allow the spindle microtubules to bind to the chromosomes. The budding yeast, *Saccharomyces cerevisiae*, undergoes closed mitosis, in which the nuclear membrane remains intact, and the spindle forms inside the nucleus. To facilitate a closed mitosis,

the yeast equivalent of the centrosome, called the spindle pole body, is embedded in the nuclear membrane throughout the yeast life cycle. Like the centrosomes, the spindle pole body duplicates at the start of mitosis; however, duplication of the spindle pole body must occur in the context of the nuclear membrane.

One of the proteins that make up the spindle pole body is Mps3, which is required for the duplication of the spindle pole body. Because of Mps3's critical function during mitosis, cells without Mps3 are unable to complete mitosis and arrest with a single spindle pole body and malformed mitotic spindle. The *MPS3* gene was thus thought to be an essential gene – without it, yeast cells cannot survive.

A Brief Lesson in Genetics

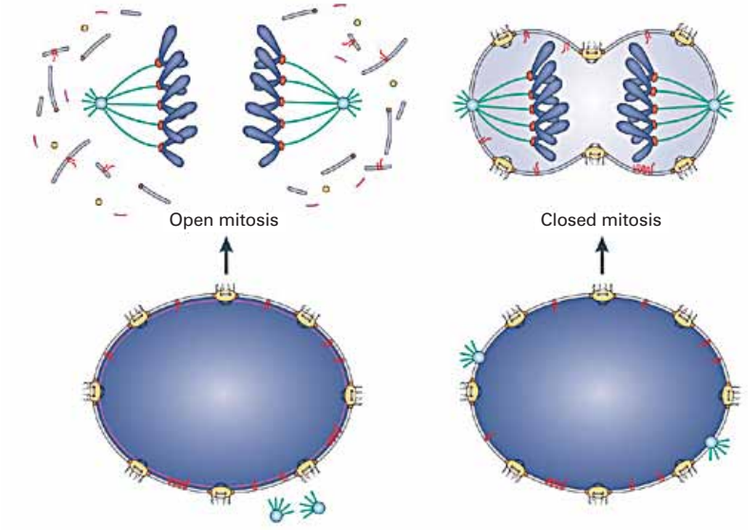
Using yeast genetic techniques, researchers can deduce whether the genes they are studying affect the same biological process. As a simple example of the concept, let us consider a table supported by four legs. If we remove one leg, the table will not fall, although it will be considerably less stable. If we restore that leg and remove another one, again the table will not fall. If we, however, remove both legs simultaneously, the table will in all likelihood fall and hit the floor.

Similarly, some biological processes can be thought of as a table supported by legs. The legs in this case represent the genes or proteins that work together to make that process happen. Removing only one gene may not cripple the biological process, but removing multiple genes may. In situations where mutating one or another gene has little effect on a cell, but mutating both genes simultaneously leads to severe sickness or death, the mutations are said to be *synthetically sick* or *synthetically lethal*. Synthetic effects are an example of a genetic interaction – it suggests that both genes affect the same biological process.

Unexpected Results

The current study, published in the November 2010 issue of *Genetics*, relied heavily on this kind of genetic analysis in order to identify new relationships between already known genes and proteins in yeast. Orna Cohen-Fix, Ph.D., and Keren Witken, Ph.D., researchers at the National Institutes of Health, were interested in a gene called *SPO7*. *SPO7* encodes a

Most animal cells undergo open mitosis, in which the nuclear membrane dissolves at the start of mitosis (on the left). Budding yeast cells undergo closed mitosis, in which the nuclear membrane remains intact (on the right). The spindle pole bodies are embedded within the nuclear membrane, and the mitotic spindle forms within the nucleus.



Adapted from Guttinger et al. Nat Rev Mol Cell Biol. *2009. 10:178-91.*

protein that regulates membrane lipids in the cell. In yeast strains where *SPO7* has been deleted, the shape and composition of the nuclear membrane are abnormal. Strangely, Dr. Cohen-Fix and Dr. Witken noticed that their genetic screens were revealing interactions between *SPO7* and genes encoding spindle pole body components.

Intrigued by this, Dr. Cohen-Fix and Dr. Jaspersen began a collaboration to investigate how the *SPO7* gene, which encodes a protein that affects nuclear membrane lipids, might be interacting with genes that encode members of the membrane-associated spindle pole body. Using genetic techniques, the team confirmed that there were indeed specific genetic interactions between *SPO7* and *MPS3*.

Further genetic experiments revealed two strange and unexpected results. First, genes encoding nuclear pore components also interacted with *SPO7* and *MPS3*. Second, although *MPS3* was thought to be an essential gene, deleting both *MPS3* and *NUP157* (which encodes a structural component of the nuclear pore) resulted in viable yeast cells. In other words, deleting components of the nuclear pore complex enabled the yeast cell to survive without Mps3 protein.

With the assistance of Rhonda Trimble, Electron Microscopy Specialist in Histology, the team used electron microscopy to carefully study the structure of the spindle pole body. They were surprised to find that, even without Mps3, the double mutant appeared to undergo normal duplication of the spindle pole body and assembly of a normal spindle. This confirmed that under certain conditions, *MPS3* turns out not to be an essential gene after all.

Take-Home Message

The team proposed two theories to explain their surprising results. First, the mutant nuclear pore complexes that resulted from deletion of *NUP157* may have affected the physical properties of the nuclear membrane in a way that somehow enabled the spindle pole body to duplicate without Mps3.

Alternatively, the spindle pole body and the nuclear pore complex may be competing for some limiting unknown factor. Deleting *NUP157* or other related genes may have resulted in this mysterious factor associating less with the nuclear pore and more with the spindle pole body, enabling the spindle pole body to duplicate without Mps3.

In this post-genomic age, when high-throughput large-scale screens are increasingly common in biological research, Dr. Jaspersen finds it refreshing that basic yeast genetics still holds the power to make new discoveries. "What was mostly used here was old-school techniques and standard yeast genetics. Old is not necessarily bad."

More studies will be required to figure out which one of the above scenarios is happening. The take-home message for the team, however, is that a change in lipid composition for the membrane can have significant effects on the cell. "We've learned in this study that the membrane actually does a lot more than just form a barrier – it isn't this inert structure," says Dr. Jaspersen. "What's in the membrane – the lipids and the proteins – can affect chromosome dynamics."

PAPER: Changes in the Nuclear Envelope Environment Affect Spindle Pole Body Duplication in *Saccharomyces cerevisiae*

JOURNAL: *Genetics*

ISSUE: November 2010

AUTHORS*: **Keren Witkin, Ph.D.**, National Institutes of Health; **Jennifer Friederichs**, Laboratory Assistant; **Orna Cohen-Fix, Ph.D.**, National Institutes of Health; **Sue Jaspersen, Ph.D.**, Assistant Investigator

**Authors' primary appointments are with the Stowers Institute for Medical Research unless otherwise noted.*

Sue Jaspersen, Ph.D., Associate Investigator, is also an Assistant Professor in the Department of Molecular and Integrative Physiology and the University of Kansas School of Medicine. Learn more about her work at http://www.stowers.org/labs/JaspersenLab.asp.

2010

Year in Review

Taking Stock

At the close of 2010, 475 people worked at the Stowers Institute. 356 people were members of the scientific staff, including:

- 20 Principal Investigators
- 3 Technology Center Directors
- 78 Postdoctoral Research Associates and Fellows
- 45 Predoctoral Research Associates

By Stephanie Huang, Ph.D.

Making a Mark

Photo by Jay Casillas

In 2010, Stowers Institute research teams continued to make discoveries meriting publication in leading peer-reviewed scientific journals – 54 articles in all. Add to that 44 reviews, commentaries, and chapters, and two books – it all makes for another successful year. Highlights among 2010's published research articles include:

- The **Si Lab** published results that suggest that ApCPEB acts as a self-sustaining prion-like protein in the nervous system, enabling it to play a role in memory formation. (February 5 issue of *Cell*)
- The **Shilatifard Lab**, in collaboration with the **Conaway Lab** and the **Proteomics Center**, identified a protein called AFF4 that associates with mutant proteins commonly found in mixed lineage leukemia and that may be a key regulator of leukemia development. (February 12 issue of *Molecular Cell*)
- The **Mak Lab** reported novel defects that result in expansion of lipid droplets in the roundworm *Caenorhabditis elegans*, contributing to the elucidation of cellular pathways that regulate fat storage. (March 9 issue of the *Proceedings of the National Academy of Sciences* and published online on February 22)
- The **Kulesa Lab** and the **Microscopy Center** showed that multicolor cell labeling and multispectral imaging enable more accurate cell tracking in the chick neural crest, an important developmental structure. (September 24 issue of *BMC Developmental Biology*)
- The **Baumann Lab** collaborated with **Bill Neaves, Ph.D., President Emeritus**, the **Microscopy Center**, and the **Reptile and Aquatics Facility** to reveal unique aspects of meiosis in parthenogenetic whiptail lizards that enable these female lizards to preserve existing genetic variation when reproducing asexually. (March 11 issue of *Nature* and published online on February 21)
- The **Yu Lab** showed that high and low concentrations of pheromones activate different subsets of neurons, helping to explain how mammalian species respond differently to different concentrations of pheromone cues. (June 2 issue of the *Journal of Neuroscience*)
- The **Gibson Lab** discovered and characterized a novel red fluorescent protein in the sea anemone *Nematostella vectensis*, defining *Nematostella* as a new model organism for studying the natural functions of fluorescent proteins. (Published online in *PLoS ONE* on July 27)
- The **Hawley Lab** provided the first direct evidence that the protein calcineurin is essential for the completion of meiosis in *Drosophila* oocytes. (August 15 issue of *Developmental Biology* and published online on June 16)
- The **Workman Lab**, in collaboration with the **Abmayr Lab**, **Proteomics Center**, and **Bioinformatics Research**, revealed that a protein complex called ATAC helps to modulate the cellular response to osmotic stress. (September 3 issue of *Cell*)
- The **Krumlauf Lab** demonstrated that the protein Wise regulates the well-studied Wnt protein signaling pathway to control tooth development, including tooth number and shape. (October issue of *Development* and published online on August 19)
- The **Blanchette Lab**, in collaboration with scientists outside the Institute, reported a novel aspect of the exon junction complex (EJC), a protein complex that assembles on messenger RNA as a consequence of splicing, or the removal of non-coding sequences called introns. (October issue of *Nature Structural and Molecular Biology* and published online on September 5)
- The **Jaspersen Lab**, in collaboration with scientists outside the Institute, revealed an unexpected relationship between the yeast protein Mps3, components of the nuclear pore complex, and lipids of nuclear membrane. (November issue of *Genetics* and published online on August 16)
- The **Rong Li Lab**, in collaboration with the **Proteomics Center**, **Molecular Biology**, and **Bioinformatics Research**, demonstrated how aneuploidy in budding yeast can cause changes in gene expression and confer a growth advantage in certain environmental conditions. (November 11 issue of *Nature* and published online on October 20)
- The **Xie Lab** published work showing how the protein Lissencephaly-1 may regulate the self-renewal state of adult stem cells. (November 16 issue of the *Proceedings of the National Academy of Sciences* and published online on November 1)
- The **Gerton Lab**, in collaboration with the **Workman Lab** and the **Proteomics Center**, uncovered the roles of two proteins, Psh1 and Scm3, in helping to ensure the presence of only one centromere per chromosome. (November 12 issue of *Molecular Cell*)

Accolades

Photo by Jay Casillas

(back row from left) Erin Guest, Caleb Bailey, Joan Conaway, Paul Trainor, Ronald Conaway, Kausik Si, Ali Shilatifard, (front row from left) R. Scott Hawley, Laurence Florens, and (not pictured) Linheng Li

- **R. Scott Hawley, Ph.D.**, Investigator, received an American Cancer Society grant, effective in January.
- **Ali Shilatifard, Ph.D.**, Investigator, received two National Institutes of Health grants, effective in March and December, and an Innovation Award from Alex's Lemonade Stand Foundation, effective in July.
- **Paul Trainor, Ph.D.**, Associate Investigator, received a National Institutes of Health grant, effective in July, was a sub-recipient on another National Institutes of Health grant from the University of Kansas Medical Center, effective in July, and was a sub-recipient on a Kansas City Area Life Sciences Institute grant from the University of Missouri-Kansas City, effective in August.
- **Joan Conaway, Ph.D.**, Investigator, and **Ronald Conaway, Ph.D.**, Investigator, received a National Institutes of Health grant, effective in July.
- **Kausik Si, Ph.D.**, Assistant Investigator, received the 2010 Hudson Prize from the M.R. and Evelyn Hudson Foundation, effective in July.
- **Erin Guest, Ph.D.**, Visiting Scientist in the Shilatifard Lab, received a Midwest Cancer Alliance grant, effective in July.
- **Laurence Florens, Ph.D.**, Head of Proteomics, was a sub-recipient on a National Institutes of Health grant from the University of California, Riverside, effective in September.
- **Caleb Bailey, Ph.D.**, Postdoctoral Research Associate in the Kulesa Lab, received a Ruth L. Kirschstein National Research Service Award from the National Institutes of Health, effective in September.
- **Linheng Li, Ph.D.**, Investigator, was elected as a Fellow of the American Association for the Advancement of Science in the Section on Medical Sciences in December.

In 2010, the following researchers left the Institute to continue their careers elsewhere:

- **Juntao Gao, Ph.D.**, Postdoctoral Research Associate, Rong Li Lab – Postdoctoral Research Associate, University of Illinois at Urbana-Champaign
- **Jie He, Ph.D.**, Postdoctoral Research Associate, Yu Lab – Postdoctoral Research Associate, Cambridge University, UK
- **Kasthuri Kannan, Ph.D.**, Research Specialist, Microscopy Center – Research Associate, Pennsylvania State University
- **Lavanya Kannan, Ph.D.**, Postdoctoral Research Associate, Bioinformatics Research / Mushegian Lab – Postdoctoral Research Fellow, American Museum of Natural History, New York
- **David Kristensen, Ph.D.**, Postdoctoral Research Associate, Bioinformatics Research / Mushegian Lab – Postdoctoral IRTA Fellow, National Center for Biotechnology Information
- **Amber Mosley, Ph.D.**, Postdoctoral Research Associate, Washburn Lab – Assistant Professor, Indiana University School of Medicine
- **Chunlei Wang, Ph.D.**, Postdoctoral Research Associate, Yu Lab – Postdoctoral Research Fellow, Georgia Health Sciences University
- **Le Zhan,** Predoctoral Researcher, Yu Lab – Graduate Student, University of Kansas Medical Center
- **Nian Zhang, Ph.D.**, Scientist, Xie Lab – Research Assistant Professor, University of Rochester Medical School

The following research technicians left the Institute in 2010 to pursue graduate degrees or continue their careers elsewhere:

- **Veronica Conaway**, Rong Li Lab – University of Missouri School of Medicine
- **Katherine Hollander**, Microscopy Center – Georgetown University School of Nursing and Health Sciences
- **Elspeth Pearce**, Abmayr Lab – University of Kansas Medical Center
- **Katherine Prather**, Kulesa Lab – Georgetown University School of Medicine
- **Morgan Romine**, Kulesa Lab – The Maxwell School of Citizenship and Public Affairs at Syracuse University
- **Laura Schaefer**, Zeitlinger Lab – Kansas State University College of Veterinary Medicine
- **Katherine Waugh**, Molecular Biology – University of Colorado, Denver
- **Ruihong Zhu**, Cytometry Facility – Managing Director Flow Cytometry, Pasteur Institute Shanghai

The following graduate students completed their Ph.D. degrees in 2010 and left the Institute to pursue further training:

- **Matthew Goering, Ph.D.**, Gerton Lab – Shawnee Mission Medical Center
- **Jason Ross, Ph.D.**, Linheng Li Lab – University of California, San Diego
- **David Scoville, Ph.D.**, Linheng Li Lab – University of Kansas Medical Center

Advancing Work, Advancing Careers

The Stowers Institute is pleased to congratulate Susan Abmayr, Ph.D., on her renewal as Associate Investigator and C. Ron Yu, Ph.D., on his promotion from Assistant Investigator to Associate Investigator, effective January 1, 2011.

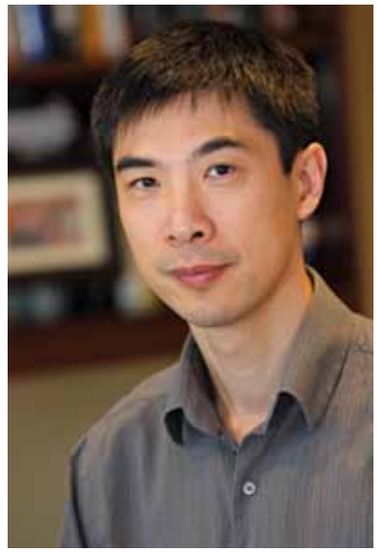
Photos by Jay Casillas

Susan Abmayr

The Abmayr Lab studies organogenesis, the process by which specialized cell types are generated and organized into functional structures. Using as a model the fruit fly *Drosophila melanogaster*, her team studies the development of larval body wall muscles, with a particular emphasis on the process of recognition and fusion between myoblasts, precursors of muscle cells. A new area of Dr. Abmayr's research focuses on *Drosophila* nephrocytes, cells that are analogous to those of the vertebrate kidney and that function in the detoxification of the insect blood system. Her work aims to elucidate signaling pathways important to muscle development and kidney function.

Dr. Abmayr joined the Institute in 2003 from Pennsylvania State University where she served as Associate Professor of Molecular Genetics. She earned a Ph.D. in Biochemistry and Molecular Biology from Rockefeller University and completed postdoctoral training at Harvard University under the direction of Dr. Tom Maniatis. In addition to her appointment at the Institute, Dr. Abmayr is also Associate Professor in the Department of Anatomy and Cell Biology at the University of Kansas Medical Center.

Learn more about her work at http://www.stowers.org/labs/AbmayrLab.asp.



C. Ron Yu

The goal of Dr. Yu's research is to understand the neural circuitry that detects, parses, and integrates sensory information. His team uses the mammalian olfactory and vomeronasal systems – which process smell and pheromone stimuli, respectively – as models to understand general principles of sensory systems. Dr. Yu hopes to shed light on the way these stimuli are represented in the brain, on the development and plasticity of the olfactory system, and on the identity and regulation of specific protein players involved in pheromone response.

Dr. Yu joined the Institute in 2005 after completing postdoctoral training in the laboratory of Dr. Richard Axel at Columbia University. He holds a Ph.D. in Molecular, Cellular, and Biophysical Studies from Columbia University. In addition to his appointment at the Institute, Dr. Yu is also on the faculty of the Department of Anatomy and Cell Biology at the University of Kansas Medical Center.

Learn more about his work at http://www.stowers.org/labs/YuLab.asp.

2010 Research Leaders

Laboratories

Robert Krumlauf, Ph.D., Scientific Director and Investigator, joined the Stowers Institute in 2000 from England's National Institute for Medical Research, The Ridgeway, Mill Hill, London, where he was head of the Division of Developmental Neurobiology. Dr. Krumlauf received a Ph.D. in developmental biology from Ohio State University.

Research Focus: Analysis of molecular pathways that regulate how the mammalian head, brain, and nervous system are built, using a variety of vertebrate model systems

Susan Abmayr, Ph.D., Associate Investigator, joined the Stowers Institute in 2003 from the Pennsylvania State University where she served as Associate Professor of Molecular Genetics. She earned a Ph.D. in biochemistry and molecular biology from the Rockefeller University and completed postdoctoral training in the Department of Biochemistry and Molecular Biology at Harvard University under the direction of Professor Tom Maniatis.

Research Focus: Molecular genetics of cell fate specification and differentiation in Drosophila*, using the embryonic development of the musculature as a model system*

Peter Baumann, Ph.D., Associate Investigator and Howard Hughes Medical Institute Early Career Scientist, joined the Stowers Institute in 2002 after completing a Howard Hughes Medical Institute postdoctoral fellowship in the laboratory of Dr. Thomas R. Cech at the University of Colorado-Boulder. Dr. Baumann received a Ph.D. in biochemistry from the Imperial Cancer Research Fund and University College, London.

Research Focus: Functional analysis of telomeres and their roles in cellular immortality and cancer

Marco Blanchette, Ph.D., Assistant Investigator, joined the Stowers Institute in 2006 from a postdoctoral position with Dr. Donald C. Rio at the University of California-Berkeley where he was recipient of a Human Frontier Long-Term Fellowship. Dr. Blanchette received a Ph.D. degree in microbiology from the Université de Sherbrooke, Canada.

Research Focus: Functional genomic analysis of the mechanisms controlling alternative pre-mRNA splicing

Joan Conaway, Ph.D., Investigator, joined the Stowers Institute in 2001 from the Oklahoma Medical Research Foundation where she was an Associate Investigator of the Howard Hughes Medical Institute and interim head of the program in Molecular and Cell Biology. Dr. Conaway received her doctorate in cell biology from Stanford University School of Medicine.

Research Focus: Analysis of the molecular mechanism and regulation of gene transcription

Ronald Conaway, Ph.D., Investigator, joined the Stowers Institute in 2001 from the Oklahoma Medical Research Foundation where he was holder of the Chapman Chair in Medical Research. Dr. Conaway received his Ph.D. in biochemistry from Stanford University School of Medicine.

Research Focus: Analysis of the molecular mechanism and regulation of gene transcription

Jennifer Gerton, Ph.D., Associate Investigator, joined the Stowers Institute in 2002 from a postdoctoral fellowship in the laboratory of Dr. Joseph DeRisi in the Department of Biochemistry and Biophysics at the University of California-San Francisco. Dr. Gerton received a Ph.D. in microbiology and immunology from Stanford University.

Research Focus: Genomic and genetic analysis of chromosome segregation and chromosome dynamics

Matthew Gibson, Ph.D., Assistant Investigator, joined the Stowers Institute in 2006 from a Jane Coffin Childs Memorial Fund postdoctoral fellowship with Dr. Norbert Perrimon at Harvard Medical School. Dr. Gibson received a Ph.D. in zoology from the University of Washington.

Research Focus: Genetic analysis of mechanisms controlling signal transduction, cell proliferation, and epithelial morphogenesis during Drosophila *development*

R. Scott Hawley, Ph.D., Investigator, joined the Stowers Institute in 2001 from the University of California-Davis where he was a professor of genetics in the Molecular and Cellular Biology section. Dr. Hawley earned a Ph.D. in genetics from the University of Washington and completed postdoctoral training as a Helen Hay Whitney Fellow at the Institute for Cancer Research in Philadelphia.

Research Focus: Investigation of mechanisms that influence how chromosomes pair and segregate during meiosis using Drosophila *as an experimental system*

Sue Jaspersen, Ph.D., Assistant Investigator, joined the Stowers Institute in 2005 from the laboratory of Dr. Mark Winey at the University of Colorado-Boulder where she was a Keck Foundation Fellow, a Helen Hay Whitney Fellow, and the recipient of a Leukemia & Lymphoma Society Career Development Award. Dr. Jaspersen holds a Ph.D. in biochemistry from the University of California-San Francisco.

Research Focus: Inner nuclear membrane protein localization and role in chromosome positioning and segregation

Linheng Li, Ph.D., Investigator, joined the Stowers Institute in 2000 from the University of Washington Medical Center where he held a faculty appointment after completing postdoctoral training in the laboratory directed by Dr. Leroy Hood. Dr. Li earned his Ph.D. in molecular and cellular biology from New York University Medical School under the mentoring of Dr. Edward Ziff.

Research Focus: Investigation of molecular and genetic pathways controlling adult stem cell development in the hematopoietic and intestinal systems using transgenic and gene targeting animal model approaches

Rong Li, Ph.D., Investigator, joined the Stowers Institute in 2005 from the Department of Cell Biology at Harvard Medical School where she served as an Associate Professor. She earned a Ph.D. in cell biology at the University of California-San Francisco with Dr. Andrew Murray and held a Damon Runyon-Walter Winchell Cancer Research Fellowship as a postdoctoral associate with Dr. David Drubin at the University of California-Berkeley.

Research Focus: Mechanism of cell polarization and cell motility, genome dynamics and cellular evolvability, and epithelial tissue morphogenesis

Ho Yi Mak, Ph.D., Assistant Investigator, joined the Stowers Institute in 2006 from a Human Frontier Science Program postdoctoral fellowship in the laboratory of

Photo by Jay Casillas

Dr. Gary Ruvkun at Harvard Medical School. Dr. Mak received a Ph.D. in molecular pathology from the Imperial Cancer Research Fund and University College, London.

Research Focus: Analysis of molecular pathways that regulate cellular fat storage in response to nutrient availability and lipid metabolism

Ali Shilatifard, Ph.D., Investigator, joined the Stowers Institute in 2007 from the Saint Louis University School of Medicine where he was a Professor of Biochemistry and Associate Director for Basic Sciences at the Saint Louis University Cancer Center. Dr. Shilatifard earned a Ph.D. in biochemistry from the University of Georgia and the University of Oklahoma School of Medicine and completed postdoctoral training as a Jane Coffin Childs Fellow at the Oklahoma Medical Research Foundation.

Research Focus: Molecular pathway of leukemogenesis

Kausik Si, Ph.D., Assistant Investigator, joined the Stowers Institute in 2005 from the laboratory of Dr. Eric Kandel at Columbia University Center for Neurobiology and Behavior where he was a Jane Coffin Childs Fellow and a Francis Goelet Fellow in Neuroscience. Dr. Si earned a Ph.D. in molecular biology from the Albert Einstein College of Medicine.

Research Focus: Role of synaptic protein synthesis in information acquisition and memory storage

Paul Trainor, Ph.D., Associate Investigator, joined the Stowers Institute in 2001 from a research position at the National Institute for Medical Research at Mill Hill, London, where he completed postdoctoral training. Dr. Trainor has a Ph.D. in developmental biology from Children's Medical Research Institute at the University of Sydney, Australia.

Research Focus: Investigation of the interactions between distinct tissues in the body and their regulation during normal development to reveal pathways that regulate normal cranial and facial development

Jerry Workman, Ph.D., Investigator, joined the Stowers Institute in 2003 from the Pennsylvania State University where he held the Paul Berg Professorship of Biochemistry and was an Associate Investigator of the Howard Hughes Medical Institute. Dr. Workman earned a Ph.D. in cell and molecular biology from the University of Michigan and completed postdoctoral training at the Rockefeller University with Dr. Bob Roeder.

Research Focus: Study of the protein complexes that modify chromatin

Ting Xie, Ph.D., Investigator, joined the Stowers Institute in 2000 after completing a Howard Hughes Medical Institute postdoctoral fellowship in the laboratory of Dr. Allan C. Spradling at the Carnegie Institution of Washington. Dr. Xie received his Ph.D. from the Joint Graduate Program in Molecular Biology and Biochemistry of Rutgers University and the University of Medicine and Dentistry of New Jersey.

Research Focus: Genetic and molecular analysis of stem cells and germ cell development in Drosophila *and mouse*

C. Ron Yu, Ph.D., Associate Investigator, joined the Stowers Institute in 2005 from the laboratory of Dr. Richard Axel at Columbia University Center for Neurobiology and Behavior where he held a National Institutes of Health Mentored Research Scientist Award from the National Institute of Mental Health. Dr. Yu earned his Ph.D. in molecular, cellular, and biophysical studies at Columbia University.

Research Focus: How olfactory sensory information is detected, integrated, and processed in the brain to influence specific innate behaviors

Julia Zeitlinger, Ph.D., Assistant Investigator, joined the Stowers Institute in 2007 from the lab of Dr. Richard Young at the Whitehead Institute for Biomedical Research at Massachusetts Institute of Technology where she was the recipient of a long-term postdoctoral fellowship from the Human Frontier Science Program. Dr. Zeitlinger earned a Ph.D. in molecular biology from the European Molecular Biology Laboratory in Heidelberg, Germany.

Research Focus: Analysis of the gene regulatory networks underlying cellular differentiation

Technology Centers

Paul Kulesa, Ph.D., Director of Imaging, joined the Stowers Institute in 2002 after completing a Burroughs Wellcome Fund postdoctoral fellowship in the laboratory of Dr. Scott E. Fraser at the California Institute of Technology. Dr. Kulesa received a Ph.D. in applied mathematics under Dr. J.D. Murray at the University of Washington.

Research Focus: Cell migration in development and cancer

Arcady Mushegian, Ph.D., Director of Bioinformatics Research, joined the Stowers Institute in 2001 from Akkadix Corporation in San Diego where he led the bioinformatics program. Dr. Mushegian earned a doctorate in molecular biology at Moscow State University and received training at the University of Kentucky, University of Washington, and with Dr. Eugene Koonin at the National Center for Biotechnology Information at the U.S. National Institutes of Health.

Research Focus: Computational analysis of genes and proteins

Michael Washburn, Ph.D., Director of Proteomics, joined the Stowers Institute in 2003 from the Torrey Mesa Research Institute in San Diego where he was a Senior Staff Scientist in Proteomics. He earned a Ph.D. in biochemistry and environmental toxicology from Michigan State University before completing a postdoctoral fellowship with Professor John Yates, III in the Department of Molecular Biotechnology at the University of Washington.

Research Focus: Quantitative proteomics and protein complex dynamics

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