Research Digest

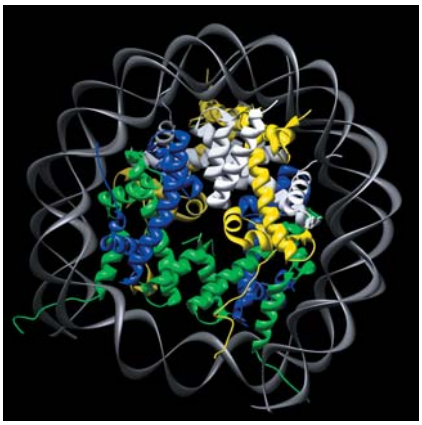
Synopses of Research Articles

Depositing a Histone That Protects Active Chromosomal Regions from Silencing

When James Watson and Francis Crick reported the structure of DNA in 1953, the mechanism of inheritance was instantly apparent. The complementary pairing of the DNA bases in the double helix, the pair famously wrote, "immediately suggests a possible copying mechanism for the genetic material." The structure helped explain one of the central problems of modern biology: how does genetic material get faithfully replicated and then passed on from generation to generation? It was long thought that DNA is the only unit of inheritance.

Since then, it's become clear that molecules of DNA are packaged into highly organized superstructures that themselves are inherited. These structures play a significant role in the regulation of genes by preventing or facilitating protein–DNA interactions. In the eukaryotic cell (a cell with a nucleus), DNA exists as long threadlike molecules—a typical human cell contains some 6.5 feet (2 meters) of DNA—that associate with a variety of proteins to form a network called chromatin. Genomic DNA wraps around specialized DNA-packing proteins called histones to form nucleosomes, which condense chromatin into chromosomes and thereby influence chromosome behavior. Chromosomes are in turn packaged in increasingly higher levels of organization, with some parts being dispersed and others condensed. The most condensed region is called heterochromatin, or silent chromatin. Gene expression is largely silent in these regions, since the proteins required for transcription can't access DNA to transcribe genes when chromosomes are so tightly packed. Other regions of chromosomes exist in an extended state, called euchromatin. This is the most genetically active state; with genes exposed, transcription can easily occur.

As chromatin shifts between these states, it influences gene expression, largely through the interactions of histones and large protein complexes that together assemble, remodel, and modify chromatin. Since proper cell function depends largely on activating the right gene at the right time, mechanisms have evolved that protect active genes from the intrusions of silencing structures like heterochromatin.



Nucleosome containing H2A.Z

Both euchromatin and heterochromatin respond to mechanisms that resist encroachments of the opposite state. One mechanism involves replacing "canonical" (that is, archetypal) histones with a histone variant. Previous work on yeast from Hiten Madhani and colleagues had shown that one histone variant, called H2A.Z, is found specifically in euchromatin and prevents silent chromatin from spreading into adjacent euchromatic regions. While researchers have characterized some of the mechanisms that deposit canonical histones onto euchromatin, they knew little about the mechanisms that deposit variant histones. In this issue of *PLoS Biology,* Jasper Rine, Hiten Madhani, and colleagues identify and characterize the function of a protein complex that helps deposit the variant H2A.Z onto euchromatin in yeast.

To investigate which proteins help direct H2A.Z to specific chromosomal locations, the authors isolated H2A.Z, along with whatever proteins were associated with it, from yeast cell extracts. They determined that 15 proteins were true binding partners of H2A.Z and that 13 of them form a complex called SWR1-Com. The largest subunit of this complex, called Swr1p, belongs to a well-known family of adenosine triphosphate (ATP)-dependent chromatin remodeling enzymes (they use the energy of ATP to power remodeling) that provide access to DNA in chromatin. Rine, Madhani, and colleagues show that protein subunits of SWR1-Com associate specifically with the histone variant H2A.Z. By comparing the gene expression profiles of yeast mutants lacking the H2A.Z-encoding gene with mutants lacking the Swr1p-encoding gene, the authors show that H2A.Z depends on the SWR1-Com protein complex to function. Most importantly, they show that SWR1-Com is required in living cells to deposit H2A.Z onto euchromatin. Interestingly, the authors note, SWR1-Com shares subunits with a histone-acetylating enzyme involved in the regulation of transcription (called the NuA4 histone acetyltransferase) and with another chromatin remodeler, which suggests that biochemical modifications of the subunits on histone "tails" may play a role in replacing H2A with H2A.Z.

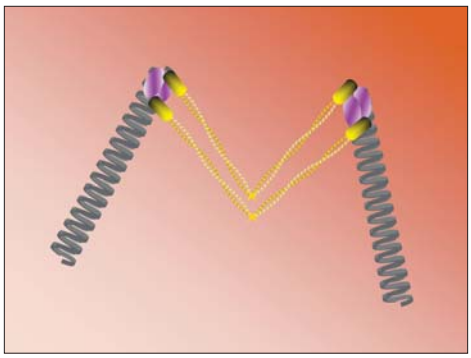
This histone–protein complex, the authors conclude, represents a chromatin remodeling machine with a novel function, revealing a new role for Swr1p-type enzymes and a novel mechanism of genome regulation. By preventing the spread of silent chromatin into transcriptionally active chromosomal regions—the result of the interaction described here—this mechanism allows the cell's gene expression program to operate with precision and on schedule. Since chromosomes can be inherited by daughter cells in this active state, such mechanisms ensure that gene expression programs essential for ongoing fundamental processes like embryogenesis and cellular differentiation proceed without interference.

Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, et al. (2004) A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. DOI: 10.1371/journal.pbio.0020131

The Mre11 Protein Is Necessary for DNA Damage Response

With billions of cells in the adult human body, all replicating and dividing in an environment laden with toxins, radiation, and free radicals, a certain amount of DNA damage is guaranteed to occur. Fortunately, all organisms have built-in checkpoints throughout the cell cycle that prevent such mistakes from propagating. At the G1 checkpoint during cell division, for example, molecules survey nuclear DNA for errors and breaks before the cell is deemed fit to undergo S phase, the DNA replication stage. If damage is found, enzymes either work to repair it or, in some cases, trigger programmed cell death, or apoptosis. But when checkpoints fail, and DNA damage is left unrepaired, disease such as cancer can result. A better understanding of these events, as provided, for example, by Vincenzo Costanzo and colleagues in this issue, will consequently lead to a better understanding of the mechanisms that give rise to cancer.

A serious form of DNA damage, called a double strand break (DSB), cuts the helix clean through—a far worse scenario than if just one strand slips free. In response to a DSB, the cell recruits a signaling protein called ATM and a three-protein complex called MRN, whose components selectively bind to broken DNA ends. A malfunction of this signal and repair pathway is dire. People who suffer from the genetic disease ataxia-telangiectasia (A-T) lack a functioning ATM molecule and therefore cannot properly handle DSBs or successfully navigate the G1 checkpoint. This condition leads to a host of problems, including abnormal chromosomes, deficient immune function, and a predisposition to cancer. A-T-like disease (ATLD), another rare genetic condition, has very similar symptoms. The only difference is that the protein missing is Mre11, a subunit of MRN. While recent work on the cellular level has indicated that MRN activates ATM, the biochemical relationship between these proteins has yet to be fully understood.



Model for Mre11 complex bridging DNA molecules

Studying these two molecules using traditional biochemical assays is difficult because knocking out the activity of these proteins is lethal to many cells. Costanzo and colleagues used a novel test system of cell-free frog extracts and found that Mre11 is necessary for both ATM activation and for the formation of large protein–DNA complexes apparently responsible for triggering the cascade of signaling molecules underlying the DNA damage response at the G1 checkpoint.

The frog extract system allowed the team to manipulate the presence or absence of Mre11 and accurately measure the response triggered by the addition of fragmented bits of DNA (simulating naturally occurring DSBs). As predicted, without a functional Mre11 protein, ATM was not activated and there was no response. By simply adding the protein Mre11 back to the mixture, the damage response was restored. But when the researchers added a mutant form of Mre11, still capable of performing its essential tasks in another stage of the cell cycle—DNA replication—the G1 damage response remained suppressed. This mutant form of the Mre11 protein lacks the C-terminal, or DNA-binding, end.

Costanzo and colleagues also found that this DNA-binding end is required for the assembly of DNA–ATM–MRN complexes in the presence of fragmented DNA and seems to direct the entire damage response. This work helps to explain the similarity between patients with A-T and those with ATLD, and hints at the formation of a large "signaling" complex that helps to orchestrate the crucial response to DBSs in DNA.

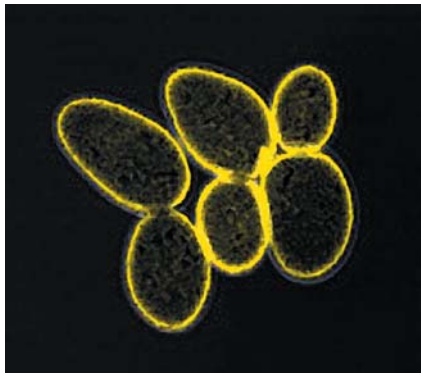
Costanzo V, Paull T, Gottesman M, Gautier J (2004) Mre11 assembles linear DNA fragments into DNA damage signaling complexes. DOI: 10.1371/journal.pbio.0020110

Dissecting the Complexities of Glucose Signaling in Yeast

An organism's survival depends on developing effective strategies for identifying and adapting to available sources of food. Even organisms as small as the budding yeast *Saccharomyces cerevisiae* can respond in a complex way to the presence of different energy sources. While yeast can metabolize many different sugars, glucose provides the highest energy yield. To achieve this energy efficiency, yeast cells rely on specialized enzymes and metabolic states that mediate glucose metabolism by sensing and responding to this sugar. The presence of glucose triggers a rapid and dramatic change in the expression of about a quarter of *S. cerevisiae*'s 5,500 genes.

Many of the cellular participants in the glucose "sense-and-response" pathway have been identified, but their exact relationships remain unknown. The gene expression response to glucose has been previously characterized through the use of a cDNA microarray, which allows simultaneous assessment of the transcriptional state of every gene. In research reported in this issue, James Broach and his colleagues at Princeton University have attempted to connect the individual components in this complex pathway by performing microarray analysis on a series of mutants (yeast strains with defects in specific proteins). They link certain portions of the response to known proteins and begin to understand how the pieces of this pathway fit together.

The researchers initially focused on two proteins, called Ras2 and Gpa2. These proteins have been previously implicated in the transcriptional response to glucose and are members of a well-established family of signaling proteins. To investigate



Ras at the plasma membrane of *S. cerevisiae*

the roles of these proteins in the response, the researchers used mutants of Ras2 and Gpa2 that could be activated on demand and then performed microarray analysis to see which genes responded to activation of these proteins. They found that even in the absence of glucose, activation of either protein induced a transcriptional profile almost identical to the profile generated in yeast exposed to glucose. This shows that the complex and dramatic transcriptional response to glucose can be recapitulated by the activation of a single protein.

The Ras2/Gpa2 pathway, however, is not the whole story. The group then went on to show that not all glucose-responsive genes are regulated in the same manner. They found that another pathway, independent of Ras2 and Gpa2, is able to elicit a portion of the transcriptional response to glucose. The partial redundancy of these pathways is a curious phenomenon and bears further investigation.

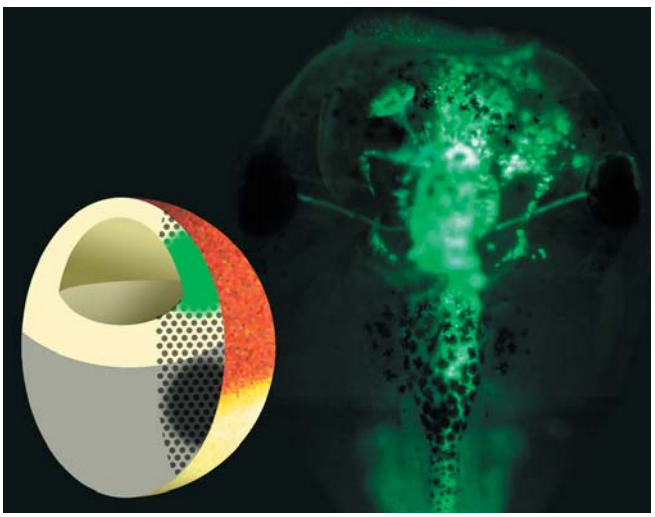
The authors have achieved an initial step in mapping the topology of the intricate signaling pathway (or pathways) involved in the response to glucose. Furthermore, the approach—that of using microarray analysis of mutants in a pathway to deduce the mechanisms of regulation—will be useful in efforts to map other complex responses in yeast as well as in higher organisms.

Wang Y, Pierce M, Schneper L, Güldal CG, Zhang X, et al. (2004) Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. DOI: 10.1371/journal.pbio.0020128

Neural Induction without Mesoderm in *Xenopus*

Formation of the central nervous system has long been thought to result from an induction process, whereby signals emanating from a portion of the dorsal endomesoderm (the inner middle layer of the developing embryo), known as the Spemann–Mangold organizer, instruct cells of the overlying dorsal ectoderm (outer layer) to become neural instead of epidermal. The Spemann–Mangold organizer was itself defined in Spemann and Mangold's seminal 1924 publication as a portion of the dorsal "vegetal" half (also known as the endodermal, or inner, layer) of a gastrulating *Xenopus* frog embryo that could induce the differentiation of a whole new axis, including a new central nervous system, when grafted into an abnormal location. (Gastrulation is the process that establishes the basic body plan of the organism as cells arrange themselves into three embryonic germ layers: the endoderm, mesoderm, and ectoderm.) From these and later experiments, the notion emerged that neural induction in *Xenopus* takes place at gastrulation and requires signals from the mesoderm. (The Spemann–Mangold organizer is itself derived from the endomesoderm.)

Now Hiroki Kuroda, Oliver Wessely, and Edward De Robertis challenge this model by demonstrating that a group of cells in the dorsal region of the prospective ectoderm is fated to become neuronal as early as the blastula stage (which precedes gastrulation) and that these cells can express their neural character in the absence of any mesodermal influence. The authors call this group of cells the BCNE (blastula Chordin- and Noggin-expressing) center, based on their previous observation that this center expresses the proteins Chordin and Noggin at the blastula stage. Chordin and Noggin are also expressed later in the Spemann–Mangold organizer and are among the key signals that mediate neural induction by the organizer. The presence of the neural inducers in blastula ectodermal precursor cells prompted the authors to test these cells' neural potential. They first demonstrated that BCNE cells normally give rise to the anterior portion of the brain, which confirms these cells' neural fate. Moreover, when cultured in vitro, BCNE cells taken from tissue begin to express neural protein markers, even when extra care is taken to prevent any contact with mesodermal precursors. It therefore appears that BCNE cells are already specified to become neural by the blastula stage, before the Spemann–Mangold organizer forms.



Blastula cells that give rise to the brain

To further demonstrate BCNE cells' independence from mesodermal signals, the authors generate embryos without a mesoderm. Having previously observed that such embryos do develop a central nervous system, Kuroda et al. now demonstrate that this intrinsic neuronal potential depends on Chordin and Noggin expression in BCNE cells. The model that emerges from these experiments suggests that neural induction begins at the blastula stage, with Chordin and Noggin signaling within the BCNE center and may later be consolidated or modulated by signals emanating from the organizer.

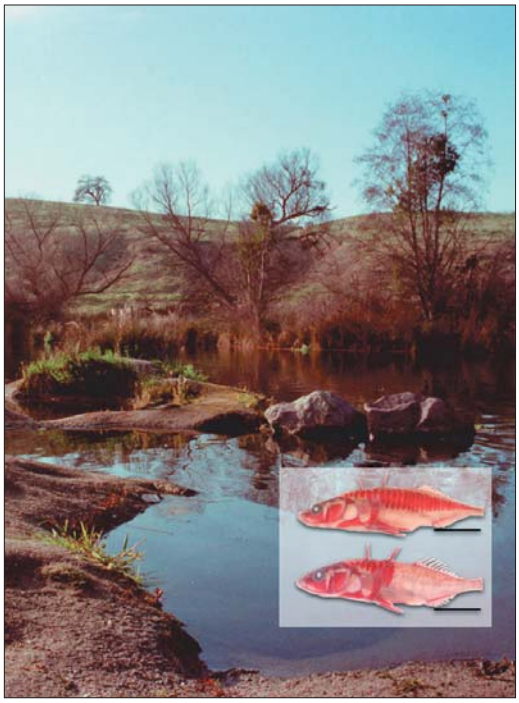
What of the endodermal portion of the Spemann–Mangold organizer? It expresses a secreted protein called Cerberus that is involved in development of the head. The authors show that abolishing Cerberus function in the prospective endoderm results in headless embryos. Complete brain removal can also be achieved by partially inhibiting Cerberus function, so long as Chordin is simultaneously inhibited in the dorsal ectoderm. It is therefore likely that while BCNE cells harbor an intrinsic neural potential, neural induction in a living organism occurs via cooperation between the germ layers.

Kuroda H, Wessely O, De Robertis EM (2004) Neural induction in *Xenopus*: Requirement for ectodermal and endomesodermal signals via Chordin, Noggin, β-Catenin, and Cerberus. DOI: 10.1371/journal.pbio.0020092

Single Locus Controls Majority of Armor Evolution in Two Populations of Sticklebacks

The astounding diversity of life—different body shapes and sizes, physiologies, and behaviors—stems from the accumulation of genetic changes through the process we call evolution. But catching a glimpse into the process of evolution at the gene level is difficult, mostly because significant changes to the plant and animal species of today happened a long time ago. Nevertheless, biologists are keen to understand exactly how evolution progresses. For example, how many genes must be altered before noticeable shifts in appearance can be seen? Is evolution the result of changes in many genes with small additive effects, or of just a few mutations that exert a strong influence?

To tackle these questions, Pamela Colosimo and colleagues turned to threespine stickleback fish, a longtime favorite model system of evolutionary biologists because of its relatively youthful evolutionary history. At the end of the last ice age 10,000 years ago, when glaciers all over the Northern Hemisphere began to melt, small populations of these originally marine-dwelling fish became trapped in newly formed lakes. There, isolated stickleback colonies adapted to new ecological conditions—different predators, food availability, water chemistry, and temperature—and now look distinctly different from their marine ancestors. One of the most obvious changes in appearance is in their body armor—they come in three distinct types, or "morphs." Marine sticklebacks are covered from head to tail with rows of tightly packed boney plates (a complete morph), while those found in freshwater lakes have fewer body plates (a partial morph) or almost none at all (a low morph). Colosimo and colleagues found that a single region of the genome is largely responsible for the dramatic changes in plate morph, and that this is true for two widely separated populations of independently evolving freshwater sticklebacks.



Complete and low armor stickleback morphs, Friant, California

To uncover the genomic regions that affect armor, Colosimo's team crossed fully armored marine sticklebacks from Japan with deep-water, or benthic, low morph fish from Paxton Lake in British Columbia, Canada. They then "mapped" the full genome of second generation offspring using 160 known genetic markers, or loci, as guideposts for distinct regions of the genome—loci that are inherited along with differences in the overall type of plating, and individual plate number and size.

The team found that one such locus explained 75% of the variation in plate morphs. Offspring that carried two alleles—versions of the gene—from their marine grandparents, genotype *AA,* were almost always fully plated. Those that inherited two copies of the allele from their benthic progenitors, *aa,* were mostly low morphs with very little plating. And *Aa* heterozygous fish (with one allele from each population) had mostly full or partial plates. Colosimo and colleagues also found three other regions in the genome that significantly affected the number and size of plates. These modifiers had an additive effect—the more benthic alleles inherited, the fewer and smaller the plates; more marine alleles caused a trend toward greater armor.

But is this genetic architecture the same for every independently evolving population of lake-bound sticklebacks in North America? Or did the geographically isolated freshwater groups loose their plates through mutations in different genes? Colosimo and colleagues mapped the genome of a population of sticklebacks from Friant, California, which is 800 miles away from Paxton Lake, and found that the same major locus seemed to be controlling plate morph there as well. Crossing a low morph from Friant with a low morph from Paxton yielded only offspring with very little armor. Further, some of the modifiers uncovered in the Paxton fish were also acting on the Friant sticklebacks. So, though these two populations of fish have been separated for 10,000 years, loss of armor in both groups probably stemmed from changes in the same genetic pathway.

Without knowing the precise sequence of these genes, it is impossible to tell exactly how and when the alleles that reduce armor arose. Small numbers of individuals with genes causing less plating could have been present in ancestral populations of marine sticklebacks when they were originally locked in newly formed lakes. Alternatively, reduced armor could have arisen independently in different lakes following isolation if, for example, some genes that control armor are predisposed to mutation, or certain armor-related mutations are more advantageous than others. But however it happened, this study clearly shows that dramatic morphological evolution can result from a small number of genetic changes. Further study of this classic system should provide a detailed picture of the genes involved, and of the molecular events that underlie morphological changes in natural populations evolving in new environments.

Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, et al. (2004) The genetic architecture of parallel armor plate reduction in threespine sticklebacks. DOI: 10.1371/journal.pbio.0020109

Small RNA Pathways in Plants

Since small RNA molecules were discovered just over ten years ago, it's become clear that these once overlooked bits of genetic material play a decidedly large role in controlling gene expression. Though typically just 21 to 24 nucleotides long, small RNAs regulate a diverse array of cellular processes, from developmental patterning and genome rearrangement to antiviral defense. They typically accomplish these tasks by targeting specific nucleotide sequences to shut down gene expression.

Found in both plants and animals, small RNAs come mainly in two classes—microRNA (miRNA) and short interfering RNA (siRNA). miRNAs arise from non-protein-coding transcripts that adopt extended "fold-back" structures, which are then cleaved by enzymes called Dicer or Dicer-like (DCL). siRNAs, on the other hand, arise from perfectly base-paired double-stranded RNA, which are also cleaved by Dicer. Some siRNAs require enzymes called RNA-dependent RNA polymerases (RdRp). miRNAs and many types of siRNAs function post-transcriptionally—that is, they affect genes that have been expressed, or transcribed, into RNA—to guide cleavage or prevent translation into protein. In plants and some animals, this post-transcriptional RNA interference (RNAi) acts as an adaptive antiviral response, among other things. siRNAs can also "silence" gene expression by altering chromatin—the DNA-protein complex into which chromosomes assemble—and preventing transcription. It is thought that chromatin silencing acts as a genome defense mechanism, guarding against potential damage from mobile genetic elements or invasive DNA (say, from a virus) by keeping genes in the tightly coiled, and thus inaccessible, "heterochromatic" state.

While much remains to be learned about the mechanisms and pathways that govern small RNAs, it's becoming clear that they add an important layer of regulation and flexibility to gene expression. Now a team led by James Carrington at Oregon State University and Steve Jacobsen at the University of California at Los Angeles demonstrates that plants have evolved multiple systems to produce distinct classes of small RNAs with specialized regulatory and defensive functions. The first generates miRNAs; the second produces siRNAs that regulate chromatin structure; and the third generates siRNAs in response to viral infections. Each system requires a unique spectrum of functions of three different DCL proteins; the siRNA systems each function in coordination with one of several RdRp proteins. The researchers propose that the expansion and subsequent diversification of these proteins, which occurred in plants but not in many animals, has contributed to the diversification of specialized small RNA-directed pathways.

***A. thaliana* at the rosette stage**

Working in *Arabidopsis thaliana,* a favorite model organism for plant biologists, Zhixin Xie et al. analyzed a series of mutants with nonfunctional *dcl* and *rdr* genes, as well as a few other mutants of interest, to determine how the small RNAs responded to loss of these proteins. Two mutations (one in a *dcl* gene and one in another gene) affected the miRNAs, either impairing their function or reducing their populations. None of the RdRp proteins had any effect on miRNAs. The researchers performed the same type of genetic analyses on siRNAs and found that a different DCL mutant caused a reduction in one class of siRNAs and that an RdRp mutant nearly eliminated these populations of siRNAs.

The diversity of siRNAs produced by the *Arabidopsis* genome reveals an important role in genome maintenance, expression, and defense, the authors conclude. Given that large numbers of siRNAs arise from highly repeated sequences—such as those introduced by viruses or mobile genetic elements—it may be that the cell senses such "invasive" sequence duplication events and enlists siRNAs to run interference by silencing these potentially damaging sequences. In this way, chromatin-associated siRNAs may offer an additional line of defense against invasive sequences, on top of that offered by post-transcriptional RNAi—a dual adaptive advantage since a fast-spreading virus or over-proliferating transposon (also known as a jumping gene) could wreak havoc on a plant population.

Whatever other roles small RNAs may play in genome regulation—they have also been implicated in regulating growth and development—their primary responsibility appears to be blocking gene expression. Whether they accomplish that by controlling chromosome activity to prevent gene transcription or by inhibiting or degrading RNA transcripts to block translation into protein, small RNAs appear to make wide-ranging contributions to the overall gene expression program of the cell.

Xie Z, Johansen LK, Gustafson AM, Kasschau KD, Lellis AD, et al. (2004) Genetic and functional diversification of small RNA pathways in plants. DOI: 10.1371/journal.pbio.0020104

Evolutionary History of a Gene Controlling Brain Size

Biologists have long known that the African great apes (including the chimpanzee, bonobo, and gorilla) are our closest relatives, evolutionarily speaking. The recent release of the chimp draft genome sequence confirms this relationship at the nucleotide level, showing that human and chimp DNA is roughly 99% identical. Given the genetic similarity between human and nonhuman primates, the next big challenge is to identify those changes in the human genotype (the genetic complement of an organism) that generated the complex phenotype (the physical manifestation of gene expression) that distinguishes humans from the great apes. For example, modern humans have larger brains and a larger cerebral cortex than both nonhuman primates and their forebears, the early hominids. Elucidating the molecular mechanisms that account for this expansion will provide insight into brain evolution.

One way to figure out which genes are involved in a physiological process is to analyze mutations in the genotype that generate an abnormal phenotype. Such efforts are easier in the relatively rare instance that one gene affects a single trait. Mutations in the *ASPM* gene cause microencephaly, a rare incurable disorder characterized by an abnormally small cerebral cortex. Since the microencephalic brain is about the same size as the early hominid brain, researchers hypothesized that *ASPM*—whose normal function is unclear—may have been a target of natural selection in the expansion of the primate cerebral cortex. Last year, researchers showed that selective pressure on the *ASPM* gene correlated with increased human brain size over the past few million years, when humans and chimps diverged from their common ancestor. Now, Vladimir Larionov and colleagues report that the selective pressure began even earlier—as far back as 7–8 million years ago, when gorillas, chimps, and humans shared a common ancestor.

MRIs of a normal individual (bottom left) and a patient with microcephaly caused by an *ASPM* mutation (bottom right). Primate skulls provided courtesy of the Museum of Comparative Zoology, Harvard University.

The researchers used a newly developed technology (called TAR-cloning) to extract specialized cloning agents in yeast (called yeast artificial chromosomes, or YACs) containing the entire *ASPM* gene, including promoter and intronic (noncoding) sequences, from chimpanzees, gorillas, orangutans, and rhesus macaques. They sequenced these YACs to determine the complete genomic sequence of the *ASPM* gene from each species. Next, they characterized sequence changes among these species, based on whether the resulting substitutions in amino acids produced changes in the ASPM protein, to determine how fast the protein was evolving. Larionov and colleagues found that different parts of the protein evolved at different rates, with the rapidly evolving sequences under positive selection (beneficial mutations were selected for, or retained) and the slowly evolving sequences under "purifying" selection (significant disruptions were jettisoned). Positive selection on genes is one important way to drive evolutionary change.

By reconstructing the evolutionary history of the *ASPM* gene, Larionov and colleagues show that the increase in human brain size—which began some 2–2.5 million years ago—happened millions of years after the gene underwent accelerated selective pressure. The *ASPM* gene, they conclude, likely plays a significant role in brain evolution. The next big challenge will be identifying the forces that preferentially acted on the human genotype to kick-start the process of brain expansion, forces that promise to shed light on what makes us human. New genomic technologies like TAR-cloning will likely accelerate this process.

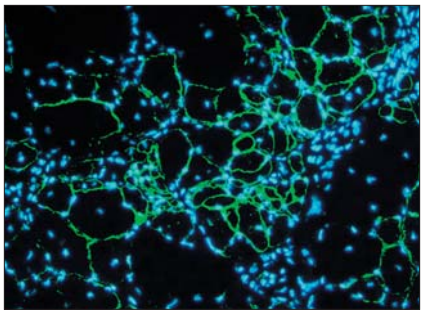
Kouprina N, Pavlicek A, Mochida GH, Solomon G, Gersch W, et al. (2004) Accelerated evolution of the *ASPM* gene controlling brain size begins prior to human brain expansion. DOI: 10.1371/journal.pbio.0020126

A Gene That Directs the Regeneration of Injured Muscle from Adult Stem Cells

If the United States' Human Cloning Prohibition Act of 2003 (H.R. 534) becomes law, American researchers practicing any form of cloning could face up to ten years in prison and a minimum $1 million fine. The bill criminalizes a research procedure, called somatic cell nuclear transfer, that involves removing the DNA from a fertilized egg and replacing it with the DNA of a body (soma) cell. While the procedure could theoretically be used to clone a human being, used therapeutically its great promise lies in yielding a renewable source of stem cells to repair and regenerate tissue damaged by disease or injury. Embryonic stem cells appear most suited to this task, but some researchers are finding that adult stem cells could perform similar duties in certain tissues. And adult stem cells, it appears, are responsive to genetic manipulation. H.R. 534 does not threaten researchers working with adult stem cells.

The precise origin of adult stem cells is unclear, though some propose that they are "set aside" during embryonic development and sequestered in mature tissue. These cells, which can make identical copies of themselves or give rise to specialized cells, serve primarily to replace damaged or injured cells. Skeletal muscle has a remarkable capacity to regenerate following exercise or injury and harbors two different types of adult stem cells to accomplish the job: satellite cells and adult stem cells that can be isolated as side population (SP) cells. Like embryonic stem cells, the adult cells commit to a certain fate once particular genes are activated.

It was thought that only satellite cells could mediate skeletal muscle regeneration until recently, when scientists found that adult stem cells not only participate in muscle tissue regeneration but also spawn satellite cells. A certain population of these stem cells, which are recognized by the cell surface proteins CD45 and Sca1 (stem cell antigen-1), is involved in normal muscle tissue repair, but is only triggered into the muscle cell development pathway by injury. The question then arises: what molecular factors turn these adult stem cells into muscle cells? Now Michael



Pax7-infected stem cells rescue dystrophin expression

Rudnicki and colleagues have shown that one gene, *Pax7,* plays a crucial role in directing the differentiation of these adult stem cells into skeletal muscle cells.

In previous studies, Rudnicki's group demonstrated that *Pax7* is required to turn adult stem cells into myogenic cells during regeneration. Here, the researchers worked with mouse models and in vitro experiments to investigate which cell populations *Pax7* targets and how the gene initiates muscle cell formation in injured tissue. They show that CD45:Sca1 cells taken from regenerating muscle in mice lacking the *Pax7* gene could not become muscle cells. And they show that by putting *Pax7* back into CD45:Sca1 cells taken from uninjured muscle, they can generate a population of proliferating myoblasts that readily differentiate into muscle cells. When CD45:Sca1 cells engineered to express *Pax7* proteins were injected into the muscles of mice lacking dystrophin (the protein defective in muscular dystrophy), the cells differentiated, forming dystrophin-expressing muscle cells in the defective muscle. This shows that engineered "donor cells" can differentiate in living tissue and help repair dystrophic muscle. When the researchers injected *Pax7* (using a gene therapy virus) into the damaged muscle of mice lacking *Pax7*, they observed the production of muscle-forming cells that not only gave rise to differentiated muscle cells, but also aided in tissue repair.

The researchers argue that these results "unequivocally establish" *Pax7* as a key regulator of muscle cell differentiation in specific populations of adult stem cells during muscle tissue regeneration. If therapeutic strategies that activate *Pax7* in adult stem cells can turn them into muscle cells, effectively replenishing injured or diseased muscle tissue, there's hope of reversing the debilitating effects of progressive muscle-wasting diseases. Though the clinical efficacy of such an approach will require intensive investigation, the results on these adult stem cells are encouraging—especially in this political climate.

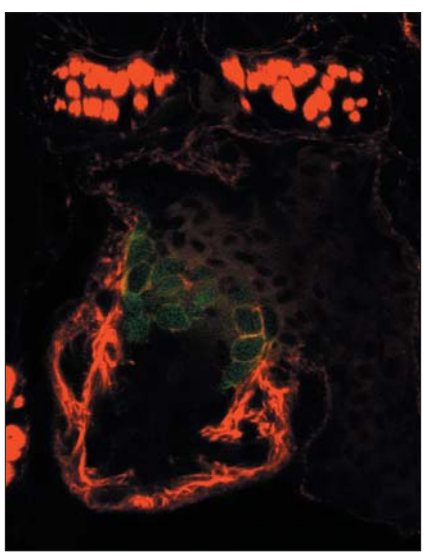
Seale P, Ishibashi J, Scimè; A, Rudnicki MA (2004) Pax7 is necessary and sufficient for the myogenic specification of CD45$^+$: Sca1$^+$ stem cells from injured muscle. DOI: 10.1371/journal.pbio.0020130

A Role for Early Cardiac Function in Cardiac Morphogenesis

The heart starts beating and pumps blood through the body long before it has achieved its mature architecture. In theory, this provides a chance for cardiac function to sculpt cardiac structure, an intriguing possibility for developmental biologists, and one of potentially great clinical import for cardiologists seeking to identify the causes of (often fatal) cardiac anomalies. In this issue of *PLoS Biology,* Thomas Bartman et al. use the powerful tools afforded by zebrafish genetics to dissect the early steps of heart valve formation. In the process, they provide evidence for a causal relationship between the early function of the heart and its final structure.

At the time of its first beat, the vertebrate heart is little more than a tube, lined on its outside by a myocardial cell layer whose contractions (the heartbeats) power blood flow, and on its inside by an endocardial cell layer, an extension of the inner wall of the connecting blood vessels. What it lacks still are valves and septae, the fibrous gates that subdivide the mature heart into atrial and ventricular chambers, and control the directionality of blood flow. These structures derive from the endocardium in a process that begins—shortly after the establishment of blood flow—with the local accumulation of endocardial cells into what are known as endocardial cushions (ECs).

The zebrafish lends itself well to large-scale genetic screens, and powerful genomic tools are now available to efficiently identify the gene affected by any mutation. The authors have used genetic screens to identify several mutations that affect early cardiac function or morphology. Heart anomalies are easy to detect in zebrafish, and can be examined in real time and in live specimens because the embryos develop outside the mother and are fully transparent. Using a fluorescent molecular marker highly expressed in the ECs, the authors narrowed in on mutations that result in valve defects, and identified a mutant they named *cardiofunk (cfk),* which was devoid of ECs. Genetic mapping of the *cfk* mutation revealed a single sequence change in a gene encoding a novel actin molecule that is most closely related to the sarcomeric actins found in sarcomeres, the contracting organelles of muscle cells. The result was surprising because contractions are not a property of endocardial cells.



tie2::GFP+ cells form the endocardial cushions

Using RNA detection assays, the authors show that the *cfk* gene is in fact expressed in the myocardium, rather than in the endocardium. It therefore appears that the inability to form ECs in *cfk* mutants does not reside in the endocardium per se, but is an indirect consequence of a myocardial anomaly.

The *cfk* mutation introduces a single amino acid change in the actin protein, and through detailed biochemical analyses, the authors show that the mutant actin is impaired in its ability to assemble into fibers in vitro. What might be the consequence in vivo? The authors note that *cfk* mutants display abnormal heart contractions prior to the development of their EC defect. Support for the notion that myocardial contractions are required for EC formation comes from the examination of *silent-heart (sih)* mutants. *sih* mutants, which lack a heatbeat, have been shown

to harbor a mutation in troponin T, one of the motors of actin contractions; the authors find that *sih* mutants also fail to develop ECs. The mechanisms linking myocardial contractions and cushion formation remain unclear. Blood flow may be a trigger, though the authors find that ECs can develop even in the presence of pharmacological compounds that abolish it. The characterization of additional mutants should help answer this question.

Valve or septal defects represent 40% of cardiac anomalies in humans. Bartman and colleagues suggest that, by analogy with zebrafish, some may result from congenital defects affecting very early myocardial function. Their work thus opens new avenues for the early detection of human cardiac malfunctions and malformations.

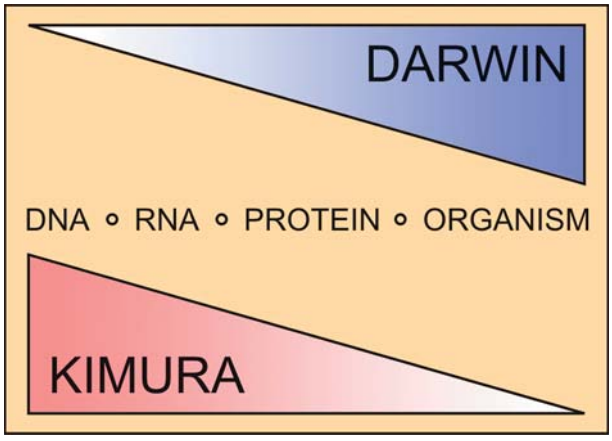
Bartman T, Walsh EC, Wen K-K, McKane M, Ren J, et al. (2004) Early myocardial function affects endocardial cushion development in zebrafish. DOI: 10.1371/journal.pbio.0020129

Random Processes Underlie Most Evolutionary Changes in Gene Expression

Are evolutionary changes in gene expression determined mostly by natural selection or by random forces? It's been some 150 years since Charles Darwin proposed that organisms adapt to their environment through the process of natural selection, yet the debate still rages, particularly at the molecular level. Darwinian selection was challenged in 1983 by the Kimura neutral theory of molecular evolution, which argues that the majority of differences in DNA (nucleotide) and protein (amino acid) sequences within and between species have only minor or no selective effect and that these differences arise through mostly random processes. Mutations at the nucleotide level occur randomly and regularly. Some of them survive through generations, resulting in "fixed" evolutionary changes between species. Two potential mechanisms can lead to the fixation of a particular change: natural selection, which favors changes that convey a selective advantage, and stochastic (random) events, such as genetic drift (the random fluctuations in genotype frequencies that occur from generation to generation in small populations).

DNA mutations can lead to changes in gene expression levels, some of which may convey a selective advantage to an organism and therefore become fixed via natural selection. But since variation is produced at the genotype level, while selection is thought to operate largely at the phenotype level (that is, the physical manifestation of the genotype), it is reasonable to expect selection to be less apparent at the level of DNA sequence, and by extension, at the level of gene expression. Microarray technology has made it possible to systematically study expression levels of thousands of transcripts (the RNA copies of DNA that are translated into amino acid sequences) and to ask whether most changes of gene expression fixed during evolution between species result from selective or stochastic processes.

To investigate this question, Philipp Khaitovich and colleagues analyzed the observed transcriptome differences among primate and mouse species as well as among various brain regions within a species. The team started out by analyzing the expression levels of some 12,000 genes in the prefrontal cortex of various primates, including humans. If evolutionary changes are caused by chance and not by natural selection, they will accumulate as a function of time rather than as a function of physical or behavioral changes in the organism. And that's what the authors found: the changes in gene expression among the species progressed linearly with time, suggesting that gene expression in primate brains evolved in large part from random processes introducing selectively neutral, or biologically insignificant, changes.



According to neutral evolution theory, the same forces determine the rate of evolution both within and between species because similar random processes are at work on both levels. Consequently, genes that vary more within species should be more likely to vary between species. Comparing the expression levels of genes according to their variation within humans, the authors showed that genes with high variation among humans changed significantly faster between species than genes with low variation among humans. The authors also compared changes observed in genes to changes observed in pseudogenes (genes that over evolutionary time acquire a mutation that renders them nonfunctional) and found no significant difference between the two, suggesting again that most expression changes have no functional significance.

While their analysis cannot exclude a role for natural selection, all the results are consistent with a neutral model of transcriptome evolution. This means that the majority of gene expression differences within and between species are not functional adaptations but selectively neutral and that we won't be able to explain species differences based on variation in gene expression in general.

In addition to examining differences in gene expression in a particular tissue between species, the authors also discuss the evolution of different tissues within a species. The human brain is composed of regions that differ in function and histology (microscopic structure). Each of these regions acquired a functional or histological difference that separated it from its sister regions at some point in our evolutionary past. The authors show that the amount of change between regions correlates with tissue-divergence times estimated by other methods. If this finding applies for other tissues within and outside the brain, it could provide a method to reconstruct the evolution of tissues within a species.

Khaitovich P, Weiss G, Lachmann M, Hellmann I, Enard W, et al. (2004) A neutral model of transcriptome evolution. DOI: 10.1371/journal.pbio.0020132

Defects in Ribosomal Protein Genes Cause Cancer in Zebrafish

To investigate the genetic underpinnings of a particular biological process, geneticists screen large collections of mutant organisms to characterize their physical defects. By comparing the genetic makeup of nonmutant (called wild-type) organisms to mutants, it's possible to tease out the genes responsible for a defective appearance, or phenotype. In a classic study in the fruitfly, Christiane Nüsslein-Volhard and Eric Weischaus bred many lines of flies with mutations that were lethal: the fly embryos died, but not before displaying a wide range of developmental defects. Since it was known that the fruitfly needed only a single wild-type copy of these genes to survive, the mutations in these "embryonic lethals" had to be recessive, meaning that both copies, or alleles, of the gene had to be mutated for the lethal defect to appear. Nüsslein-Volhard and Weischaus's work revealed many such recessive genes crucial to early development and earned them a Nobel Prize.

Among the model systems for studying development, the zebrafish has become prized because its transparent embryo develops outside the mother's body. The zebrafish has helped biologists identify many genes involved in embryogenesis and, because it's a vertebrate animal, has become a valuable resource for identifying genes involved in human disease. Now, a team led by Nancy Hopkins of the Massachusetts Institute of Technology, has created over 500 lines of zebrafish with lesions in key embryogenic genes and used them to identify a group of genes that predispose the fish to cancer, with some surprising results.

All of the 500 lines created by the researchers carried a recessive embryonic lethal mutation; for about 400 of the lines, mutations in 300 distinct genes were identified as the cause of the embryonic phenotype. During the process of cultivating some of these mutant lines, the Hopkins team noticed that an abnormally large percentage of fish experienced early mortality (in some cases, over 50% compared to the 10%–15% seen in nonmutant fish), while the surviving fish in these lines developed large, highly invasive malignant tumors; both phenotypes persisted over successive generations. The tumors resembled malignant peripheral nerve sheath tumors (MPNSTs) that have been found in other fish species as well as in mammals. Suspecting that these mutant lines had elevated rates of cancer, the researchers investigated the genetic makeup of the fish and discovered to their surprise that each line was heterozygous for a mutation in a different ribosomal protein gene *(rp)*—that is, each line carried one healthy version and one defective version of a different *rp* gene. These proteins are components of ribosomes—the massive molecular complexes within cells that mediate protein synthesis—and are essential for embryonic development.

Zebrafish tumors caused by mutation of a ribosomal protein gene

All of the *rp* mutations, the researchers report, either reduced or eliminated expression of the corresponding *rp* gene. In the case of "classic" tumor suppressor genes, the wild-type allele must be lost for the defective allele to set the stage for cancer. Here, the wild-type allele appeared to remain intact in the tumor cells, implicating the proteins as "haploinsufficient" tumor suppressors—a reduction from two gene copies to one functional copy seems to be enough to increase the risk of cancer. Apart from the mutations in *rp* genes, the authors also found a loss-of-function mutation in a gene (called *NF2*) that acts as a tumor suppressor in mammals—establishing the soundness of this approach for identifying mammalian cancer genes.

While these experiments do not explore how these mutations lead to cancer, the results suggest that some shared, ribosome-associated function allows these genes to act as tumor suppressors and that disrupting this function somehow leads to tumor formation. Though it's not clear what distinguishes the 11 *rp* genes whose mutations caused cancer from the five other *rp* genes whose mutations did not, the authors raise a number of possibilities for future study. And given the high degree of conservation of genes and pathways among vertebrates, it's likely that *rp* mutations also raise cancer risk in humans. Together, these results demonstrate that the tiny freshwater workhorse of developmental biology has a promising future as a model system for human cancer.

Amsterdam A, Sadler KC, Lai K, Farrington S, Bronson RT, et al. (2004) Many ribosomal protein genes are cancer genes in zebrafish. DOI: 10.1371/journal.pbio.0020139