

Synopsis of Research Articles

Finding Sigma-Controlled Promoters

DOI: 10.1371/journal.pbio.0040013

For bacteria, a change in the environment often causes physiological stress. Bacteria cope with that stress by altering the expression of suites of genes, and manufacturing new proteins, which may allow the cell to repair damage or protect itself in the future. This stress-induced gene expression response is often mediated by proteins called sigma factors. Sigma factors bind to the gene-transcribing machinery and direct this machinery to the promoter sites of the target genes. Identifying the binding sites for sigma factors that direct stress responses thus provides an important window on understanding how bacteria behave. Unfortunately, sigma factor binding sequences (otherwise known as promoters) vary from gene to gene, and current identification methods are tedious and prone to error. In a new study, Virgil Rhodius, Carol Gross, and their colleagues describe a novel model that finds these sites quickly and accurately. Their results show that in *Escherichia coli* bacteria, the main targets for one type of sigma factor, called sigma factor E, are genes that increase production of cell envelope components (inner membrane, periplasm, and outer membrane).

The authors first found sigma factor E sites the hard way—by comparing gene expression in two strains of *E. coli* that differed in their intrinsic level of sigma E-induced activity. They looked for genes whose expression in the high sigma E activity strain differed most from genes in the low sigma E activity strain, and then searched upstream of these genes for promoters containing these sigma sites (the region to which the transcription machinery binds). This method, called expression profiling, led them to 28 genes. This formed a “starter set,” which could be used to make their model.

By determining the nucleotide sequences and spatial arrangements that were most common at these sites, Rhodius et al. constructed a “position weight matrix,” a prediction tool with which to discover and analyze putative sigma E sites on other genes. Applied to the entire genome of bacterium *E. coli* K-12, the matrix identified 553 potential sites, which included 27 of the 28 sites identified through expression



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***Escherichia coli* (visualized with transmission electron microscopy, above) was used as a model system to predict the regulatory DNA targets of sigma factors, bacterial proteins induced by stress. Image: CDC/Elizabeth H. White, M.S.**

profiling. However, most of these sites were likely to be false positives. A series of increasingly stringent selection rules was then applied to eliminate those sites that were likely to arise by chance alone, whittling the list down to 39, including 24 of the original 28 sites. Of these 39, the authors confirmed that 37 were actual sigma factor E sites. Using a variety of other screening methods, they determined that the K-12 genome actually contained a total of 49 sigma E binding sites.

So how good are these results? A predictive model such as this is judged by

two measures: sensitivity and precision. Sensitivity, the ratio of validated predictions (“hits”) to total actual sites, indicates how well the model finds true positives. Precision, the ratio of validated predictions to total predictions (hits plus misses), indicates how well the model screens out false positives. A model that claimed that every sequence was a promoter would indeed identify all the real ones, and have a sensitivity of 100%, but the rate of false positives would make the model useless. Similarly, a model so conservative that it only made predictions guaranteed to be right would

have a precision of 100%, but might predict so little as to be of no use either.

The Rhodius et al. model has a sensitivity of 37/49, or 76%, and a precision of 37/39, or 95%, in *E. coli* K-12. The average of these two represents the total performance, or accuracy, of the predictive model, and was 85%, considerably better than previous models.

Finally, the authors used their results to predict the genes activated by sigma E in response to stress in a variety of bacteria. They found that the genes most commonly activated by sigma E primarily affect the integrity of the outer membrane, through promoting the synthesis, assembly, and homeostasis

of the two major components of the membrane: lipopolysaccharide and proteins called porins. Other regulated genes promote pathogenic behavior of the bacteria. Experimental validation of the predictions in two different species of bacteria indicated that the model performed well, having a precision of about 75%. Improved ability to find such genes and to understand how they are activated has clear potential for reducing the burden of bacterial infections.

—Richard Robinson

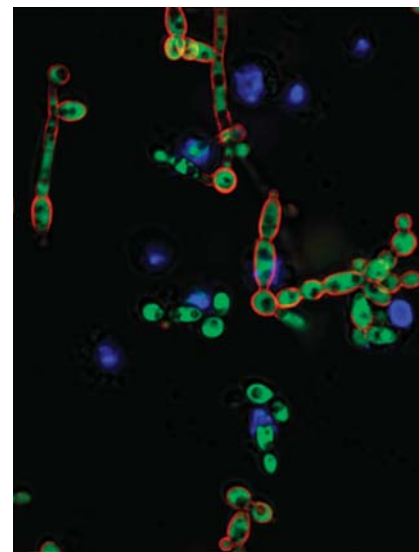
Rhodius VA, Suh WC, Nonaka G, West J, Gross CA (2006) Conserved and variable functions of the σ^E stress response in related genomes. DOI: 10.1371/journal.pbio.0040002

Fly Genes That Help Devour a Fungal Parasite

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Faced with a world full of potentially harmful bacteria, fungi, and viruses, multicellular organisms have evolved efficient innate mechanisms to combat infection soon after a pathogenic exposure. Many aspects of this innate response are shared by organisms as diverse as flies and humans, pointing to a common and ancient origin for these innate defenses. While humans and other vertebrates possess adaptive immunity—which can recognize billions of different pathogens and mount better responses with each exposure to a pathogen—flies and other invertebrates must rely only on innate immunity.

As early as 1882, Élie Metchnikoff discovered that cells in starfish and water fleas ingested and destroyed microbes as part of the immune response. (The Russian zoologist and microbiologist would receive the Nobel Prize for his discovery.) Only recently have scientists realized how many different organisms use this strategy, called phagocytosis, to protect themselves. When a pathogen is detected, signaling pathways within the phagocytic cell reconfigure the cytoskeleton and send out pseudopodia protrusions that engulf the pathogen. While these morphological and structural changes have been well described, few studies have outlined the cellular components driving this process. In a new study by Shannon Stroschein-Stevenson et al., the labs of Patrick O’Farrell and Alexander Johnson turned to the fruit fly, *Drosophila melanogaster*—a well-established model organism for both genetics and innate immunity—to



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***Drosophila* cells engulf the human pathogen *Candida albicans*, tagged with green fluorescent protein.**

explore the phagocytosis of the fungus *Candida albicans*, a widespread human pathogen.

Using the S2 cell line, which is derived from flies and shares many properties with the plasmatocytes that perform phagocytosis in the fly, the authors used RNA interference (RNAi) to conduct a global search for genes related to phagocytosis. They identified several genes specifically dedicated to dispatching *C. albicans*, then focused on one gene whose function in the fly was unknown. The gene, called *Macroglobulin complement related (Mcr)*, is closely

related to human proteins that activate what’s known as the complement cascade, an ancient mechanism that flags pathogens for subsequent recognition by phagocytic cells. The authors show that Mcr is closely related to four fly proteins (members of the thioester protein [Tep] family), and that these proteins act on different pathogens, functioning as part of a “primitive complement system” that targets specific pathogens.

Stroschein-Stevenson et al. first mixed fluorescently tagged *C. albicans* with the S2 cells and observed the S2 cells ingest the fungal cells, confirming that the cell line can phagocytose the fungus. Next, they screened the cells for phagocytosis defects using RNAi; RNAi disrupts the function of a target gene by using short lengths of double-stranded RNA (dsRNA) with complementary sequences to that gene to force the degradation of the gene’s messenger RNA and prevent its translation into protein. The authors used a library of over 7,000 dsRNAs corresponding to most of the fly’s conserved genes to screen for phagocytosis gene candidates. After treating the S2 cells with dsRNAs, they mixed the cells with the fluorescently tagged fungus to visualize the effects on phagocytosis.

The initial screen flagged some 400 dsRNAs that decreased fungal phagocytosis. A few more screens excluded dsRNAs that were likely either false positives or indirectly involved, leaving 184 dsRNAs that impaired phagocytosis. The screen identified many known phagocytosis-related genes that had been picked up in earlier screens (for example, the cytoskeletal protein actin and its various regulators, which help form the active membrane protrusions required for phagocytosis), but it also turned up many other genes not previously implicated in the process.

To distinguish genes specific to *C. albicans* from those with a broader role in phagocytosis, the authors repeated the screen with two different “challengers,” *Escherichia coli* and latex beads. Most of the 184 dsRNAs impaired phagocytosis of all three targets, but only a few specifically disrupted *C. albicans* phagocytosis, including the dsRNA for Mcr. Additional experiments confirmed Mcr’s specificity for *C. albicans*. Since Mcr is closely related to a family of four fly Tep proteins, the authors tested whether each protein is required for the phagocytosis of three

distinct pathogens: *C. albicans*, *E. coli*, and *Staphylococcus aureus*. Again, impaired phagocytosis of *C. albicans* occurred only when Mcr was disabled. (Adding Mcr proteins to the S2 growing medium restored the cells' ability to phagocytose *C. albicans*.) *E. coli* phagocytosis was reduced only with TepII disruption, and *S. aureus* phagocytosis slowed only with TepIII silencing.

The authors go on to show that S2 cells secrete Mcr, which then binds to *C. albicans* (but not to a closely related fungus) and promotes phagocytosis. Altogether, these results show that the

fly's innate immune system dispatches specialized proteins to destroy specific pathogens. The 184 genes identified in this screen should prove a valuable resource for investigations into the cellular components of an ancient defensive strategy. And with the visual screen described here, even researchers without the latest automated equipment can get started right away. —*Liza Gross*

Stroschein-Stevenson SL, Foley E, O'Farrell PH, Johnson AD (2006) Identification of *Drosophila* gene products required for phagocytosis of *Candida albicans*. DOI: 10.1371/journal.pbio.0040004

Diverse Pollination Networks Key to Ecosystem Sustainability

DOI: 10.1371/journal.pbio.0040012

As animal extinctions continue at the rate of one every 16 years, it's unclear how declining biodiversity will disturb ecosystem dynamics. Of special concern are the pollinators, essential players in the reproductive biology of plants, the earth's primary producers. Millions of years of evolutionary coadaptations lie behind the perfect pairing of pollinator proboscis anatomy with plant flower structure, as well as the mechanisms plants use to attract reproductive assistants to their food rewards. Agave plants emit musky aromas that attract lesser long-nosed bats to nectar stores within their flowers, for example. As the bats travel from flower to flower, pollen collects and then falls from their fur, facilitating cross-pollination.

These mutually beneficial relationships are sometimes so specialized that the loss of one species threatens the existence of the other, raising troubling questions about the likely consequences of declining diversity in pollination networks. In a new study, Colin Fontaine et al. tackled this question by experimentally manipulating plant and pollinator interactions under natural conditions. The authors found strong functional relationships between different pollinators and plant communities, with the highest plant community sustainability associated with the most diverse group of pollinators. These findings suggest that loss of biodiversity in pollination networks may threaten the persistence of plant communities.

For their study, the authors chose plants with easy and harder access to food rewards—three open-flower and three tubular-flower species—

and insects with short and longer mouthparts—three syrphid fly and three bumblebee species. In the spring of 2003, Fontaine et al. set up 36 plant communities in nylon-mesh enclosures in a meadow 80 kilometers (about 50 miles) southwest of Paris, after sterilizing the soil to destroy seeds and pathogens. They planted 30 adult plants in each plot at the same density, and then captured and released local pollinators into the cages during the flowering season (June–July 2003, 2004). To test all the possible plant–pollinator combinations, the authors set up three

plant treatments (open flowers, tubular flowers, and both flower types), then applied three pollination treatments (flies, bees, and both insects) to each plant treatment.

A month after the first pollination treatments, the authors tallied all the fruit on each plant, then randomly selected five fruits per plant (excepting one plant species from each group that would have required harvesting the fruit) to estimate seed production per plant. During the seedling season, they totaled the plants and the seedlings to measure plant population and reproductive success. Pollinator identity determined fruit production, with bee-pollinated plants most productive, and the different plant groups responded differently to the two pollinator groups. As expected, short-mouthed syrphid flies pollinated only open flowers, while bees pollinated both plant types. As a result, tubular flowers produced far fewer fruits with syrphid pollinators while open-flower fruit production remained the same regardless of pollinator. Fruit production increased along with both plant and pollinator diversity. Seed production was a bit more complicated. Though bee-pollinated open flowers produced fewer seeds per plant than those pollinated by syrphids, higher fruit production compensated by producing



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Loss of biodiversity in pollination networks may threaten the persistence of plant communities.

more seedlings. Fruit production increased with pollinator diversity.

As for long-term effects on plant reproductive capacity and success, tubular plant communities had fewer plants at the seedling stage than open-flowered plants (and even fewer when pollinated by syrphids). The plant species number and total plant number increased when both pollinator groups were present, and were highest with maximum plant and pollinator diversity. Seedling production showed a similar pattern: mixed plant communities treated with both pollinators yielded the most seeds.

What happened? Not surprisingly, the pollinators stuck to their preferred

plant: syrphids visited mostly open flowers, and bees visited mostly tubular flowers. Bees can pollinate open flowers but prefer tubular flowers when they have the choice, suggesting that bees may not fill a void left by a different pollinator. The presence of both pollinators allowed more appropriate pairings between insects and flowers—each performing a complementary role—leading to increased pollination efficiency and plant reproductive success.

While the study offers an admittedly pared down view of pollination networks, it demonstrates the value of studying the functional effects of pollination networks

in the field. These results show that losing a species affects plant–pollinator communities, and that such losses may ultimately trigger further reductions in biodiversity, possibly reverberating through the food chain. With as many as 70% of plant species dependent on animal pollinators and at least 82 mammalian pollinator species and 103 bird pollinator species considered threatened or extinct, this is sobering news. —*Liza Gross*

Fontaine C, Dajoz I, Meriguet J, Loreau M (2006) Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. DOI: 10.1371/journal.pbio.0040001

Thriving Community of Pathogenic Plant Viruses Found in the Human Gut

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What some people won't do for science. In the early 1980s, Barry Marshall was convinced that *Helicobacter pylori*, not stress, caused stomach ulcers and inflammation. To convince the skeptics, he drank a straight broth of *H. pylori*, and promptly developed severe gastric distress. In October, he won the Nobel Prize for Physiology or Medicine (along with Robin Warren) for his efforts.

Since Marshall's experiment, it's become clear that a wide variety of bacteria, viruses, and parasites cause gastroenteritis, and that many other uncharacterized pathogens likely cause stomach flu as well. With some 100 trillion microorganisms calling the human gut home, pathogens typically represent a slim (though often raucous) minority. Bacteria, by far the most abundant resident, mostly aid digestion. Viruses in the gut come in good (bacteriophages can aid digestion and control invasive pathogens) and bad (RNA viruses) varieties. Many viral gastroenteritis pathogens were identified by analyzing patients' fecal samples, but the viruses wouldn't grow in test tubes, forcing scientists to look for other ways to generate enough viruses for study. Amazingly, they found volunteers to ingest infected stool filtrates.

Thankfully, the tools of metagenomics offer a better way. In a new study, Tao Zhang, Yijun Ruan, and their colleagues used biomolecule filtration methods and genomics to capture and characterize the global community of RNA viruses living in the human gut. The



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The human gut appears to harbor infectious strains of the pepper mild mottle virus (seen infecting peppers, above), suggesting that humans may serve as vector for certain plant viruses. Image: UF/IFAS Pest Alert Web site/Pamela Roberts

authors set out looking for pathogenic enteric viruses, but found very few human viruses. Instead, they found a “large and diverse community” of plant RNA viruses. The most abundant of these viruses infects vegetable crops, a finding that suggests that humans likely contract the virus from food—and, most surprisingly, that humans may transmit the virus to plants.

For their study, Zhang et al. collected three fecal samples from two healthy adults in Southern California (one volunteer supplied a second sample six months later), used filtration methods to isolate the viral particles, and then constructed three libraries (one corresponding to each sample) of nearly 37,000 sequences for analysis.

Of more than 33,000 known sequences, about 75% resembled viruses, sorted into 42 different species. (The rest resembled sequences from Bacteria, Archaea, and Eukarya domains.) Only about 3% of the viral-like sequences resembled animal viruses, while 97% resembled plant viruses. Twenty-four out of 35 plant viruses identified infect commercial crops, including fruit, vegetables, and tobacco.

The most abundant plant virus, called pepper mild mottle virus (PMMV), infects a wide variety of sweet and hot peppers. When the authors compared the genetic sequences of the various PMMV strains, and then compared the sequence variation of a specific PMMV gene from all the strains, they found significant divergence at both the species and gene levels. This variability, the authors explain, suggests that PMMV exists as a diverse, dynamic population in the human gut.

So how did PMMV enter the gut? Zhang et al. enlisted three more volunteers, and tested all the food they ate two days prior to providing a fecal sample. PMMV was found in their food and at exponentially higher levels in their feces. A random test of pepper-based foods in Southern California found evidence of PMMV in three of 22 samples (none of the healthy-looking peppers tested positive). To control for local effects, the authors expanded their study to Singapore, and found PMMV in human volunteers as well as in some of the pepper-based offerings collected from food stalls. Humans not only carry this plant pathogen, we help

transmit it: when Zhang et al. inoculated Hungarian wax peppers with human-borne PMMV, every one of the plants developed a PMMV infection. Since humans can transmit the virus, other animals probably can, too—suggesting that farmers with chronically infected crops might want to test any animal manure they use.

Though it's unclear how the plant viruses manage to persist inside the human gut—do they co-opt intestinal cells or use bacteria to reproduce?—the stability of PMMV suggests that it might serve as a target to help deliver vaccines or treatments aimed at intestinal

disorders. What about the enteric viruses Zhang et al. originally sought? Human viruses may escape detection in healthy individuals, the authors explain, pointing to the next logical step: soliciting donations from patients with symptomatic gastroenteritis. Using the same global approach outlined here, the authors may well identify a host of other viral causes of gastric disorders. —*Liza Gross*

Zhang T, Breitbart M, Lee WH, Run J, Wei CL, et al. (2006) RNA viral community in human feces: Prevalence of plant pathogenic viruses. DOI: 10.1371/journal.pbio.0040003

Getting an Evolutionary Handle on Life after Reproduction

DOI: 10.1371/journal.pbio.0040016

When Richard Dawkins famously called organisms “throwaway survival machines” that exist solely to preserve the genes that made them, critics balked at the specter of genetic determinism. But Dawkins’ selfish genes derive straight from classical evolutionary theory that says life exists to reproduce, and that natural selection should act on any traits that increase reproductive success. Since many animals live beyond their fertile years, biologists have searched for evolutionary clues to this extended lifespan. What role, if any, does natural selection play in the evolution of the postreproductive lifespan?

For natural selection to shape the twilight years, postreproductive females should contribute to the fitness of their offspring or relatives, a hypothesis called the “grandmother effect.” Such contributions require that organisms spawn helpless offspring or live in extended groups where postreproductive females help raise the young. Though many mammals, including lions and baboons, rear dependent young and operate within complex social groups, studies have found no evidence of a granny effect, and females mostly live just long enough to care for their last born. For nonsocial animals that spawn independent young, extended lifespan is associated with good nutrition and the absence of disease and predators. While historical records and demographic analyses offer support for an adaptive granny effect in humans—which some biologists have offered as a possible explanation for the existence of menopause—few



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Analysis of the causes of variation in post-reproductive lifespan indicates that the evolution of lifespan in guppies is due to selection during their reproductive stage.

studies have experimentally tested for signs of selection in the evolution of a postreproductive lifespan.

In a new study, David Reznick, Michael Bryant, and Donna Holmes expand on their ongoing investigations of the life history of guppies confronting different predatory threats in Trinidad. Individuals facing different mortality threats should evolve different adaptations in their life histories, such as age at first reproduction, investment in reproduction, and patterns of senescence, including declines in reproduction. Since guppies are livebearers that provide no postnatal maternal care, Reznick et al. predicted the populations would show no differences in postreproductive lifespan—which is what they found. Though overall lifespan varied among the populations, these variations stemmed from differences in time

allotted only to reproduction.

Postreproductive lifespan, in contrast, showed no signs of being under selection, and appeared to be what the authors called a “random add-on at the end of the life history.” Random or not, this is the first demonstration of a postreproductive lifespan in fish.

Reznick et al. raised a second-generation laboratory brood of wild guppies taken from high- and low-predation streams at two locations in the mountains of Trinidad. (The high-predation sites harbor predators that frequently prey on guppies. Low-predation sites are found in the same streams, above waterfalls that exclude predators but not guppies.) A high- and low-predation site was sampled from each site, and feeding was manipulated to reflect food availability in the wild (fish in low-predation environments typically eat less and weigh less than fish in high-predation environments). Females were mated once a week until they produced offspring, and were mated again after each brood (when copulation is most likely).

The authors measured growth rate, body size, interbrood interval, and litters per lifetime for each population, and divided each individual’s lifespan into age at first birth, reproductive phase, and postreproductive lifespan. Guppies from high-predation localities gave birth sooner than those from low-predation sites; they also reproduced over a longer period and were much older when they stopped reproducing. To estimate postreproductive lifespan, Reznick et al. determined whether the time between last birth and death significantly exceeded the time needed to spawn another litter (calculated as a threshold, since interbrood intervals varied for each individual). About 60% of individuals lived beyond the time they would have been expected to produce another brood. While the authors found no differences in the probability that any particular group would enjoy an extended postreproductive lifespan or that an individual would stop reproducing before dying, they did find that the probability of experiencing an extended postreproductive lifespan increased along with the length of reproductive lifespan. Thus, even though postreproductive lifespan has no direct effect on fitness, it is linked to a component of life history that does.

Altogether, these results provide the first experimental confirmation that evolution works selectively on those aspects of life history that directly affect fitness. These findings also refute the suggestion that fish may experience little or no reproductive senescence based on evidence that they continue to produce eggs as adults. It's an open question whether postreproductive lifespan can influence fitness enough to be under selection. But in a field

dominated by investigations into the origins of human menopause and extended lifespan, the authors make a strong case for using experimental comparative analyses of other species to gain an evolutionary perspective on the human condition. —*Liza Gross*

Reznick D, Bryant M, Holmes D (2006) The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). DOI: 10.1371/journal.pbio.0040007

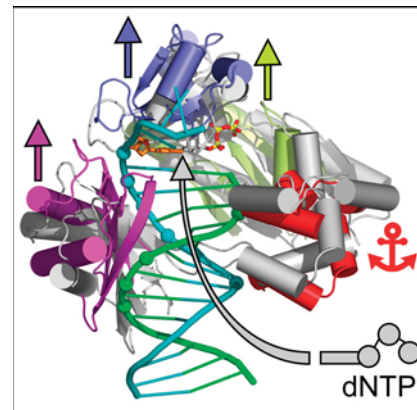
Righting the Wrongs: Structural Insights into Replicating Damaged DNA

DOI: 10.1371/journal.pbio.0040032

Every organism's blueprint for life is encoded in the order of the building blocks of its genome. These building blocks consist of four DNA nucleotides, or bases: cytosine (C), guanine (G), adenine (A), and thymine (T). Whenever a cell divides, it must duplicate its genome, and it must do so with high fidelity to maintain genome integrity. Replication is assisted by DNA polymerases. After binding to the DNA being copied (the template strand), a polymerase lines up a complementary base to the template base (C with G and A with T), then adds that base to the growing strand of nucleotides, called the primer strand. The polymerase then moves to the next position along the template DNA.

This process is further complicated by the continuous battle all cells wage against a multitude of mutagens. The result of mutagenic damage, such as radiation, can include oxidized DNA modifications, which have been linked to increased risk of cancer. Oxidative stress produces oxidized nucleotides—the most common of these is 7,8-dihydro-8-oxoguanine (oxoG), an oxidized form of guanine—which sometimes persists in DNA as a lesion. The cell is then faced with the challenge of working out how to replicate and maintain genomic integrity in spite of this anomaly.

Fortunately, the cell is armed with a toolbox to combat such situations. One of these tools, the Y-family polymerases, can bypass DNA lesions. Beyond knowing that these polymerases had this capability, not much was known about the details of the translocation mechanism, until now. In a new study, Olga Rech Koblit, Dinshaw Patel, and colleagues report a detailed insight into the mechanisms by which Dpo4, a member of the Y-family polymerases, bypasses a DNA lesion. To



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In response to DNA injury, the polymerase Dpo4 inserts a nucleotide, and three of its protein domains (light green, blue, magenta) shift upward (their prior position is shown in gray), while the fourth domain anchors Dpo4. This “bypass replication” maneuver allows DNA replication to proceed error-free.

do this, they solved crystal structures of the polymerase at different stages of association with an oxoG-modified DNA template during its replication. These structures act as snapshots of this polymerase as it progresses through lesion recognition in a pre-nucleotide insertion complex to actual nucleotide insertion, and finally, to the post-insertion complex, indicating that lesion bypass has taken place. These structures helped reveal the translocation mechanics of the bypass polymerase during a complete cycle of nucleotide incorporation that could be compared with what was already known for replication polymerases.

Rech Koblit et al. initially determined which nucleotide was most readily inserted opposite an oxoG during the

Dpo4-mediated replication process. To do this, they measured how commonly each of the four different nucleotides is inserted at this position, and the enzyme kinetics (the efficiency and speed with which the polymerase manages to carry this out) associated with each different nucleotide. What they saw was, reassuringly, that Dpo4 preferentially inserts a cytosine (dCTP) opposite the oxoG. Dpo4 is then able to continue extending beyond the lesion, facilitating error-free bypass.

The authors next concentrated on the incorporation of a dCTP opposite oxoG in their crystal structures. Structurally, Dpo4 and other Y-family polymerases have four domains: palm, finger, thumb, and little finger. This hand formation enables the protein to fit around the DNA being replicated. In the three different structures solved, Rech Koblit et al. observed how the domains moved relative to one another and identified any key parts of Dpo4 that enabled this lesion bypass. In particular, they identified two amino acids (arginines 331 and 332) in Dpo4 that play a critical role in forming hydrogen bonds formed through attraction of the positive charge of hydrogen with the nearby negatively charged phosphate group (part of the backbone of DNA) of the oxoG. With respect to the individual domains, they saw that as the dCTP inserts opposite oxoG, the little finger domain that contacts DNA phosphate groups shifts by one nucleotide step. During the next step, when dCTP is chemically bonded into place opposite oxoG, the thumb domain-phosphate contacts move along by one nucleotide. Thus, the little finger and thumb domains do not move at the same time but rather in a stepwise manner, tracking the template- and primer-strand translocation separately.

Such accumulated knowledge about DNA repair mechanisms, such as error-free lesion bypass by Dpo4, may eventually lead to cancer therapeutic approaches to reduce DNA lesions. In the meantime, such depth of mechanistic insight gleaned from these structures can lead us to wonder anew at the cell's capacity to produce such innovative solutions to the day-to-day problems it encounters. —*Emma Hill*

Rech Koblit O, Malinina L, Cheng Y, Kuryavyi V, Broyde S, et al. (2006) Stepwise translocation of dpo4 polymerase during error-free bypass of an oxoG lesion. DOI: 10.1371/journal.pbio.0040011

A Master Switch? *etsrp* Directs Blood Vessel Development in Zebrafish

DOI: 10.1371/journal.pbio.0040017

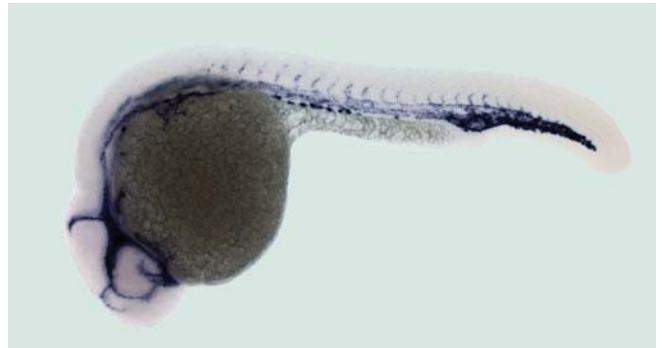
As the primary distributor of nutrients and oxygen to cells throughout the vertebrate body, the circulatory system provides life support to the body's tissues and organs. Consequently, the cellular and developmental signals that control development of this critical organ system, composed of the heart, the blood vessels, and the blood cells they contain, are the subject of intense study. Medical researchers also find it valuable to know the signals that control blood vessel development because solid tumor masses depend on infiltrating blood vessels to grow.

Circulatory system development proceeds similarly in all vertebrates, with the heart and aorta beginning to form early on (about the point at which recognizable structures like the head begin to form). Specialized cells called angioblasts create blood vessels that bud off from the aorta and branch out to form the rest of the animal's vasculature. With its transparent embryos that develop outside the mother, the zebrafish is a favored model organism for circulatory system development.

Zebrafish sporting a mutation called *cloche* (a French word referencing the mutant animal's bell-shaped heart) lack both blood vessels and blood cells. This discovery produced an important insight: the loss of two different cell types with one mutation suggests that both types of cells may arise from a common precursor at some point in early development. But until now, no single protein has been identified that differentiates the hemocytes that are destined to become blood cells from the angioblasts destined to become blood vessels. In a new paper, Saulius Sumanas and Shuo Lin describe a gene called *etsrp* that is specifically required for blood vessel development.

The authors had originally discovered *etsrp* in a screen they conducted to find genes whose expression is altered by the *cloche* mutation. To learn what exactly *etsrp* might be doing during development, the authors first looked for clues in its sequence by comparing it to the sequence of similar known proteins, the Ets family of transcription factors. The chromosomal location of *etsrp* suggested it had arisen as a result of a genetic duplication of the founding member of this family, the *ets1* gene (thus its name, which stands for "Ets1-related protein"). It's been known for some time that Ets family transcription factors play many roles in the development of the circulatory system by binding DNA and controlling the expression of genes critical to the development of circulatory system cell lineages, suggesting that *etsrp* may also be involved.

With this information in hand, the authors set out to investigate where and when *etsrp* is expressed during zebrafish development by looking for the presence of *etsrp* mRNA in developing embryos. They found *etsrp* mRNA expressed early in development in tissues that eventually give rise to blood vessels; later on, they saw *etsrp* mRNA expressed in more mature blood vessel structures throughout the animal. These findings suggested that *etsrp* might be involved in the designation of these structures during development. To see whether *etsrp* is required for blood vessel development, Sumanas and Lin disrupted the expression of *etsrp* in developing embryos. Interestingly, loss of *etsrp* resulted in the absence of blood vessels in the developing animal, even though the animals were able to make blood cells normally; the embryos' blood cells remained clumped at their formation site next to the yolk extension, rather than entering circulation. It appeared there



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The transparent zebrafish embryo helped researchers identify a putative master gene controlling blood vessel development.

just weren't any blood vessels around for the blood cells to move through. In support of this conclusion, Sumanas and Lin could not detect evidence that any body cells expressed the surface proteins normally associated with blood vessel identity. Therefore, expression of these markers appears to be dependent on the presence of *etsrp*.

If the expression of blood vessel-specific markers is dependent on *etsrp*, is it also true that *etsrp* is sufficient for expression of these markers? The authors found that artificially causing the overexpression of *etsrp* in early embryos resulted in the inappropriate expression of blood vessel-associated proteins in cells that normally do not express them. Finally, since *cloche* mutants also lack expression of blood vessel-specific markers (in addition to lacking blood cell-specific markers), the authors wondered whether artificially expressing *etsrp* in *cloche* mutants could restore the expression of markers associated with the development of blood vessels. Indeed, they found that *etsrp* could restore the expression of the blood vessel-specific marker, *flk1*, in *cloche* mutant embryos. Taken together, these data indicate that *etsrp* is both necessary and sufficient for blood vessel development in the zebrafish, lending important insights into our understanding of circulatory system development.

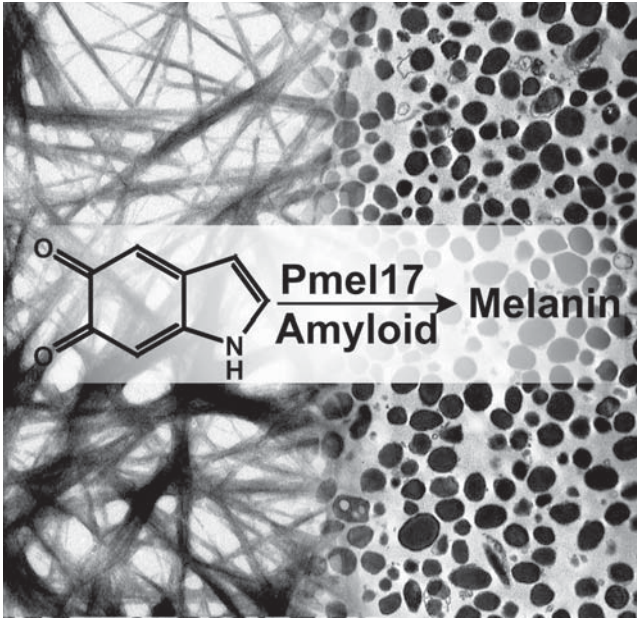
—Caitlin Sedwick

Sumanas S, Lin S (2006) Ets1-related protein is a key regulator of vasculogenesis in zebrafish. DOI: 10.1371/journal.pbio.0040010

The Unfolding of Amyloid's True Colors

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What do neurodegenerative diseases and suntans have in common? Scientists at the Scripps Research Institute have found an intriguing molecular connection. In neurodegenerative disorders such as Alzheimer and Parkinson disease, proteins aggregate into specific fibrous structures (called a cross- β sheet) to form insoluble plaques known as amyloid. Because amyloid accumulation can be highly toxic to cells and organisms, often leading to neurodegeneration, therapeutic strategies for treating such protein-conformation disorders involve targeting and reducing amyloid formation and accumulation. But in a new study in *PLoS Biology*, Douglas Fowler, Atanas Koulov, and colleagues present evidence that the amyloid structure may play



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A native, nontoxic mammalian amyloid structure, generated by the protein Pmel17, helps accelerate synthesis of melanin.

a normal role in mammalian cells. Amyloid fibrils are present in melanin-producing cells in great abundance, the authors show, where they help synthesize the sunburn-fighting pigment, melanin.

These pigments are synthesized in organelles called melanosomes, which reside in specialized skin cells (melanocytes) and the eyes (retinal pigment epithelium), to produce and traffic pigments for coloration, ultraviolet protection, and chemical detoxification. Melanosome biogenesis proceeds via a specialized pathway, related to the pathway producing a broad range of “housekeeping” organelles, including lysosomes, known for engulfing and cleaving, or lysing, proteins. Fowler, Koulov, and colleagues isolated melanosomes from retinal pigment epithelium taken from cows’ eyes, and probed them for different protein compositions. Though the authors suspected that melanosomes might contain amyloids (based on previous reports that melanosome proteins resisted denaturation, a property of most amyloid fibers), they were surprised to find the organelle loaded with fibrillar amyloids. They visualized the amyloids primarily by using fluorescent molecules exhibiting selective binding to the characteristic amyloid cross- β sheet conformation, a fluorescent microscopy method long used by pathologists to diagnose protein-conformation disorders.

Which protein contributed to the alarming abundance of the amyloid structure in melanosomes? Several clues pointed to the glycoprotein Pmel17, a critical component of melanosome biogenesis, according to genetic and biochemical data. During melanosome biogenesis, Pmel17 lyses into two fragments, one called M α that is sequestered into a membrane-bound compartment of the melanosome and another that is degraded. After confirming that the amyloids were comprised of M α fibers, the authors tried to make M α fragments fold into amyloid in a test tube. They showed that a purified, nonaggregated M α (which they called recombinant rM α) folds into amyloids remarkably quickly. When Fowler et al. compared the rate

at which rM α forms amyloids to that of other well-known amyloids—A β and α -synuclein, which are implicated in Alzheimer and Parkinson disease, respectively—they found that rM α amyloid production was at least four orders of magnitude faster. The authors offer the intriguing hypothesis that by rapidly folding into the amyloid cross- β sheet structure, M α avoids generating the toxic intermediates that are very common in pathogenic amyloid formation.

Finally, Fowler et al. satisfy a burning question: are M α amyloid fibers serving a function in melanin synthesis? After reconstituting components of the melanin biosynthetic pathway in vitro, they showed that adding rM α results in a 2-fold increase in melanin production (as does adding other amyloids like A β and α -synuclein). Perhaps more importantly, the M α amyloid fibrils bind and orient the highly reactive organic melanin precursors, mitigating the cellular toxicity observed when M α amyloid production is halted by mutation.

The authors also raise the intriguing idea that, given the propensity for many proteins to form amyloid fibrils, this conformation may be another physiologically important protein fold found in cells. To differentiate the biologically functional amyloid from pathogenic amyloids, the authors suggest using the term “amyloidin.” Although the common involvement of amyloids between melanin synthesis and protein conformation disorders is most surprising, future research into the differences between amyloid formation in these processes may hold the key for understanding diseases including Huntington, Parkinson, and Alzheimer disease. Because melanosome biogenesis is a tightly regulated process, a deeper understanding of the mechanisms that allow the Pmel17 M α fragment to avoid the toxic stage of amyloid formation could provide considerable insight into which aspects are missing when proteins misfold. —*Jami Milton Dantzer*

Fowler DM, Koulov AV, Alory-Jost C, Marks MS, Balch WE, et al. (2006) Functional amyloid formation within mammalian tissue. DOI: 10.1371/journal.pbio.0040006

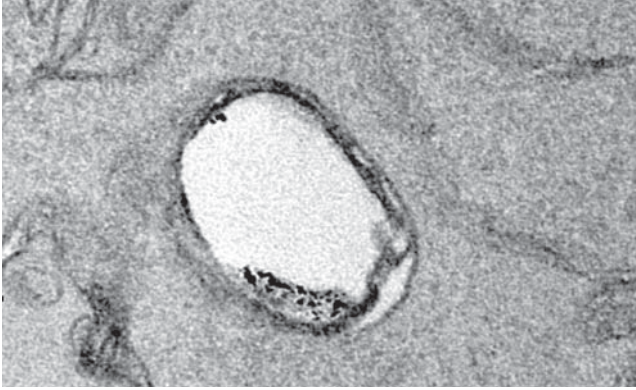
Inside a Killer: Immune Signals May Promote Vascular Growth

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“Natural killer cells”—their very name chillingly evokes their primary function as the hit men of the body’s immune system. But according to a new study by Sumati Rajagopalan, Eric Long, and their colleagues, these killers may also play a part in ensuring the success of a pregnancy. And the signal that provokes this unexpected action is itself unusual, acting not from the surface of the cell, but from within it.

Natural killer (NK) cells are a type of white blood cell. NK cells circulate in the blood stream and are also residents in a few tissues, including the uterus, where they are the primary type of white blood cell. On their surfaces, they carry a variety of receptors, which sense the exterior environment and trigger a host of internal responses. When activated, NK cells are cytotoxic—they kill target cells, such as virus-infected cells and tumor cells—and secrete soluble signaling molecules that help mount immune responses to infection by parasites, bacteria, and other invaders.

NK cell receptors bind to proteins on the surfaces of other cells. One type of NK cell receptor, called killer cell immunoglobulin-like receptor (KIR)2DL4, binds to the protein



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A natural killer cell receptor called KIR2DL4 stimulates an immune signaling pathway from inside an intracellular vesicular structure (shown above).

human leukocyte antigen (HLA)-G, whose precise role is unknown and which occurs in both membrane-bound and secreted (soluble) forms. Interestingly, HLA-G is primarily found on trophoblast cells, a type of embryonic tissue that invades the uterine lining to support the developing fetus.

The response of NK cells activated by KIR2DL4 is unusual: they release cytokines, immune chemicals that trigger actions in other cells. But this cytokine release, unlike that associated with other receptors, is not accompanied by cytotoxicity.

To understand the role of KIR2DL4-bearing NK cells in the uterus, and how they might respond to HLA-G signals, the authors examined the KIR2DL4/HLA-G interaction in detail. By activating the receptor with an antibody (to ensure that only KIR2DL4 would be triggered), they first showed that cytokine secretion could be triggered only when the antibody was soluble, not when bound to a solid surface. This behavior immediately suggested that the interaction between the two doesn't end at the membrane, since if it did, the immobilized antibodies would have sufficed to activate the cells.

They next showed that KIR2DL4 is brought into the cell by endocytosis, an energy-requiring process that cells use to carry membrane-bound molecules to the interior. They found that KIR2DL4 was present in endosomes, the vesicles formed by endocytosis. KIR2DL4 was found primarily in so-called early endosomes, but much less so in later stages, when the vesicle is being prepared for merging with a lysosome, the cell's disposal system.

What is KIR2DL4 doing in the early endosome? Rajagopalan et al. found that the receptor binds to the soluble, secreted form of HLA-G, and that HLA-G is carried with it into the endosome. Here, the complex triggered cytokine production and NK cell signaling. KIR2DL4 that could not reach the endosome could not trigger cytokine production. Therefore, the authors suggest that KIR2DL4 signaling happens from within the endosome, not at the cell surface. The cytokines produced are known to be active not only in inflammatory reactions but also in angiogenesis, or formation of new blood vessels.

Endosomes have emerged recently as important signaling compartments, and these results support and extend that understanding. On the cell surface, the receptor senses the environment for soluble ligand; once internalized into endosomes, the membrane-bound receptor interacts with signaling molecules within endosomes to set off a cascade of reactions, ultimately leading to protein production. The authors suggest that internal signaling may increase the fidelity of the desired response by avoiding the many possibly conflicting signals arising from other receptors at the surface during cell-cell interactions.

The results presented here also suggest some intriguing ideas regarding how the immune system responds to the developing embryo. Within the uterus, the embryo represents a special challenge, since it is composed of cells bearing partly foreign (that is, paternal) genetic material that must not only be tolerated but allowed to intimately intertwine with the mother's tissue to develop a new blood supply. This remodeling of the vascular system is partly under immune system control. The angiogenic cytokines triggered by KIR2DL4 activation may support this process.

Further support for this idea comes from recent observations that a higher level of soluble HLA-G promotes higher rates of successful pregnancy, while reduced HLA-G correlates with higher rates of preeclampsia, a condition caused by insufficient vascular remodeling during pregnancy. Additional study of the KIR2DL4/HLA-G signaling pathway may lead to a better understanding of this potentially fatal complication of pregnancy, and better ways to prevent it. As the production of soluble HLA-G can also be induced in certain cell types, including tumor cells, the KIR2DL4/HLA-G signaling pathway may also serve additional functions, unrelated to pregnancy. —Richard Robinson

Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der Meer A, et al. (2006) Activation of NK cells by an endocytosed receptor for soluble HLA-G. DOI: 10.1371/journal.pbio.0040009

Jumping Genes Cross Plant Species Boundaries

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In the early 1950s, legendary plant geneticist Barbara McClintock found the first evidence that genetic material can jump from one place to another within the genome. The variegated kernels of her maize plants, she determined, resulted from mobile elements that had inserted themselves into pigment-coding genes, changing their expression. McClintock's mobile elements, or transposons, moved over generations within a single species. More recently,

another form of genetic mobility has been discovered—genetic information can sometimes be transferred between species, a process called horizontal gene transfer. While horizontal genetic transfer occurs most commonly in bacteria, it has been detected in animals as well. Most transfers between higher animals involve the movement of transposons. Horizontal transfer can also occur between the mitochondrial DNA of different plant species. Until now, however, no one had

found evidence for horizontal transfer in the nuclear DNA of plants.

In a new report, Xianmin Diao, Michael Freeling, and Damon Lisch studied the genomes of millet and rice, two distantly related grasses that diverged 30–60 million years ago. While the two grasses show significant genetic divergence from accumulating millions of years of mutations, they carry some transposon-related DNA segments that are surprisingly similar. The authors conclude

that these sequences were transferred horizontally between the two plants long after they went their separate ways.

Transposons of the class identified by Diao et al. typically consist of a variable length of DNA that codes for one or more enzymes flanked by repeating sequences called terminal inverted repeats (TIRs). These repeats can bind to each other to form a “lollipop” that is easily excised from the DNA strand, carrying the rest of the transposon along with it. Plant genomes are rife with transposons, many of which are relatively passive. Transposons from the “Mutator” family in maize, however, are especially active, frequently causing mutations as they insert themselves into new positions in the genome. They perform this jump with assistance from the two proteins they code for, a transposase and a helper gene.

DNA from many species of plants contains several families of cousins of the Mutator transposons. These “Mutator-like elements,” or MULEs, code for a protein similar to the transposase, as well as the TIR sequences. Diao et al. identified 19 distinct MULEs in the DNA of various species of millet (genus *Setaria*), and compared these with the rice genome sequence, which was published in 2002. They compared the sequence similarity of these MULEs to that of other proteins that are also conserved in the same species for which sequences are available. Strikingly, they observed much higher sequence similarity between the MULEs from millet and rice than is typical for transposons. The greater similarity of the MULE DNA is easily explained if it jumped somehow, horizontally, between the species, but



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A genome-wide analysis of millet (above) and rice revealed the first clear evidence of horizontal gene transfer in plants.

there could be alternative explanations. The match could have arisen without horizontal transfer, for example, if the MULE DNA had been under positive selection, as typically happens for protein-coding genes that confer some survival or reproductive benefit. In such cases, natural selection tends to preserve the integrity of these sequences.

To test for signs of selection, the researchers looked at regions of the MULE DNA that don't appear to code for protein. The similarity between these noncoding regions in millet and rice MULEs was just as high as for the coding regions, even though selection probably doesn't influence them. Even within the coding sections, “synonymous” mutations—which don't change the protein sequence and so are not prone to selection—showed few differences between these elements.

Another explanation for the low divergence of the rice and millet MULE

sequences could be that they occur within a genomic region that, for whatever reason, experienced lower than average mutation rates. If this were the case, sequences adjacent to the elements should also show reduced variation. The authors tested this alternative hypothesis with the help of maize, which has more genomic sequence available than millet, by comparing genes flanking MULE regions in rice with evolutionarily conserved sequences in maize. The sequences did not show the similar degree of reduced variation predicted for below-average mutation rates.

Since neither selection nor low mutation frequency can explain the similar DNA between the grasses, the authors conclude, a transposon must have carried it between millet and rice long after these species diverged. Interestingly, the authors also found similar sequences in bamboo, raising the question of how common horizontal transfer may be between plant species. Given that plant mitochondrial genes appear “particularly prone to horizontal transfer,” the authors note, “it is remarkable that these results represent the first well-documented case of horizontal transfer of nuclear genes between plants.” But as researchers begin to explore the growing databases of plant genomic sequences, they can determine whether this finding constitutes an anomaly—or points to a significant force in plant genome evolution. —Don Monroe

Diao X, Freeling M, Lisch D (2006) Horizontal transfer of a plant transposon. DOI: 10.1371/journal.pbio.0040005