Stress: An evolutionary mutagen

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The fundamental conflicts in Western literature—person vs. nature and person vs. person—shape protagonists into more-evolved characters by the ends of their stories. This is no less true in biological evolution, where the environment and competing organisms shape a species' form and behavior over vast time scales, and thus its likelihood of adaptation and survival. Specific examples of variation and selection are relatively easy to come by in the natural or laboratory worlds. However, what has been much harder to find are the biological pathways, discrete molecular mechanisms that govern these broad evolutionary concepts. This is because, unlike self-aware literary characters and their responses to life's stresses, the evolution of a species' genome is constrained by a fundamental ignorance—they must be random mutations that generate the variation upon which selection occurs. In PNAS, Cappucci et al. (1) reveal a key insight that shows how a genome can seemingly intentionally respond to stress and pass those favorable adaptations on to its young.

Conrad Waddington and the Need for Adapative Evolution

The essence of the problem they solve is about "direction" and speed of evolutionary change. Two historic figures loom large over the theoretical concepts of the work being reported in this paper. First is Conrad Waddington, who famously coined the term "epigenetic" to explain the then- (and now-) mysterious "connection" between genotype and phenotype. He wondered how a phenotype "unfolds" from a DNA sequence (2). How exactly are long necks, fast running, thumbs, growling, or self-awareness encoded in DNA? Waddington knew the answer to this question must accommodate his and others' empirical and experimental observations of living and changing populations. One of the most vexing concepts for Darwinian evolution was how environmentally induced changes to an organism's shape or behavior—changes that affected the soma, the body, of the organism in question—found their way back into genotypes in the germ plasm so that the new, better-suited forms would be heritable. The famously ridiculed and rejected "Lamarckianism" (although see ref. 3) was disproven. However, it clearly happened, and it happened a lot. The problem was illustrated by Waddington's example of callosities, chest pads of thickened skin upon which ostriches would rest when sitting. Nobody doubted that callouses could develop in response to such sitting, and in all likelihood they did originally arise through a protoostrich's behavior. However, modern baby ostriches hatch with them already present. The rules of Darwin dictate that random mutations must have occurred to create callosities, but nobody could demonstrate any evidence that evolution is now actively experimenting by generating callosities randomly (or ever did), only to be removed by selection when they were not helpful. It seemed as clear as day that callosities developed from the sitting then were transferred to the offspring. This concept—that environmentally relevant characteristics were real—had to be reconciled with the apparent blindness of Darwinian processes and their mutation → form → selection paradigm.

Waddington well knew that the rules of Mendel dictated that callosities must be encoded in the germ plasm, whence sperm and eggs develop, in order to be passed on. However, he also knew that the germ plasm itself has never experienced the discomfort of sitting without a callosity, nor would a genome have the wherewithal to recognize its discomfort and direct a callosity. Much of Waddington's work was on this theoretical question. He proposed a theoretical solution he called "canalization," which posited that cryptic variants existed in the genome but were somehow masked by the action of other genes (4). Those variants, and the structures they encoded, were revealed at times of stress. Then, upon appearance of those structures, they could be selected and "assimilated" into the genome, that is, increased in allele frequency to benefit the entire population. In this way, the soma (the body experiencing the environment) does not communicate its needs to the germ plasm. Waddington's proposal could explain ostrich callosities, and probably most other new or altered forms, and it did so within

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the confines of Darwinian and Mendelian rules. Even so, it leaves one feeling a little cold. Why would those variants exist in a population? Do millions of such incipient genetic structures exist, quietly waiting? What keeps them there? What covers them up? Why could they not be seen, even in laboratory conditions when they were being looked for? Something about assimilation still felt intentional.

Barbara McClintock and the Need for Accelerated Evolution

The second scientist whose work must be briefly described to understand the significance of Cappucci et al.'s (1) work is Barbara McClintock. In her Nobel acceptance speech, and later, McClintock pointed out that "there are . . . responses of genomes to unanticipated challenges that are not so precisely programmed. The genome is unprepared for these shocks [environmental stresses, in her case]. Nevertheless, they are sensed, and the genome responds in a discernible but initially unforseen manner" (5). She perceived the same important question that Waddington did—how genomes change in response to the environment. In the course of her experiments on maize, she saw occasional mutations arise, and given the large sample size she and her contemporaries were capable of observing, she had a good idea about the rate of spontaneous mutations. However, she noted that under stressful conditions her maize had a much higher mutation rate. Ultimately, this led her to discover "jumping genes," best known now as transposable elements (6). Members of this class of "selfish DNA elements" are capable of excising or copying themselves and moving about the genome, landing in gene bodies, within the regulatory regions that control a gene's expression and so on. No prokaryote or eukaryote is free of them, and they tend to accumulate to great number: The human genome is made up of perhaps 70% transposable elements. How in the world has this ongoing internal attack not driven all life to extinction? The answer is because transposable elements are kept in check by a number of mechanisms in cells, including piRNAs, short fragments of RNAs associated with a class of proteins called argonauts (the specific argonaut here is piwi). This exquisite system involves the formation of a "library" of transposable elements encoded in an organism's genome, a codex of all of the transposable elements the organism has ever encountered, a reference to which all other sequences can be compared (7). If a cell produces an RNA transcript (necessary for transposable elements to move) that matches sequences from its library, the RNA is targeted for degradation, preventing the transposable element from doing damage. The piRNA system is limited to the germ plasm, where its components are loaded into eggs and sperm; mismatches between a species' piRNAs and the transposons within other species are responsible for the sterility of many interspecific hybrids. So, the connection between stress, transposons, and evolutionary changes (here, incipient speciation) was suspected, but a molecular mechanism was elusive—until 2010 (8).

The Modern Synthesis: Stress-Activated Transposable Elements

Specchia et al. (8), including Maria Pia Bozzetti and Sergio Pimpinelli, demonstrated that the hsp90 gene (in *Drosophila*, the organism they used, this is called hsp83) was necessary for piRNA biogenesis. Without it, the library could not be read, and offspring were unable to keep silent the transposons they carried. What was originally thought of as a mechanism of canalizing cryptic variation (9) was instead a component to tamp down the mutagenicity of transposable elements. Their work, then and subsequently, has shown that the "cryptic" variants of Waddington did not exist prior to heat shock stress but were induced by it, caused by de novo transposable element mobilizations (10).

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The problem remained that hsp83/90 is ubiquitous. It is expressed at all times and in all tissues of an organism's life, where it seemingly never stops functioning. Although a very satisfying connection to transposon biology, and one that was a clear sign of being on the right track, hsp83/90 did not fit the one critically needed characteristic: It was not responsive to stress.

Cappucci et al. (1) now show that another heat shock protein, a member of the hsp70 class, is the key positive regulator of stress-induced transposon mobilization. What is surprising, and important, about this observation is the properties of the factors involved. hsp70 is a heat shock-inducible gene (hsp70 stands for heat shock protein of 70 kDa), which functions as a molecular chaperone, refolding those proteins in the cell thermally disrupted by the heat shock conditions. They are utterly necessary to survive heat shock. They also are induced by other cellular stresses.

Cappucci et al. (1) show that upon induction hsp70 acts by displacing a similar (but not heat shock-induced) protein, hsc70, from the AGO3-containing piRNA complex in the germ line. AGO3, and the piRNA complex in general, is necessary for the repression of transposable elements. The inactivation, and the corresponding increase in transposable element activity, lasts for almost a week before the piRNA complex can be rebuilt. Also, of course, being in the germ line, defects in piRNA biogenesis concern the eggs and sperm, and thus the entirety of the descendants. Eggs and sperm produced during this time are devoid, or at least diminished, for transposable element control. These eggs and sperm are presumably hypermutated by the action of transposable elements.

Critically, their experiments did not merely break piRNA biogenesis. Rather, they identified a component that naturally disrupts it. In so doing, these investigators have uncovered a profound evolutionary mechanism for potentiating possibly genome-damaging, and possibly species-saving, hypermutation. The idea that evolution may be sped up, potentiated in times of stress, is not entirely new. Of course, it was Charles Darwin who first articulated not just that selection occurs but that it thrives in times of environmental stress. Barbara McClintock showed the same and hypothesized a link to transposable elements. This paper in PNAS finally provides a complete molecular mechanism: from heat shock sensation to increased mutation rate, the de novo creation of canalized alleles, a mechanism for assimilation, and a prime example of the evolution of evolvability. The work of Cappucci et al. (1) therefore provides a discrete and testable set of hypotheses that strikes at the core of these classical evolutionary concerns, as well as the biology of human diseases.

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