GENETICS

Tales from the crypt(ic)

Neutral mutations can breathe life into evolutionary adaptation

By **Jessica A. Lee**[1] and **Christopher J. Marx**[2]

Adaptation through natural selection requires inherited changes in an organism's phenotype. However, neutral or "cryptic" mutations—changes in genotype that do not affect phenotype—can influence adaptation outcomes, because genotype-to-phenotype mapping is inherently dependent on context. The phenotypic consequence of a mutation might change as a result of interactions either with other mutations in the genome (epistasis) or with the physical environment [a genotype-by-environment (G×E) interaction]. On page 347 of this issue, Zheng et al. (1) demonstrate that the accumulation of mutations that yield neutral changes in a protein promotes faster adaptation in an environment selecting for a new function, and that this effect requires the combined impact of epistasis and G×E interactions.

The impact of neutral mutations on adaptation is often framed from the phenotypic, rather than the genotypic, point of view; an ancestral phenotype that remains unchanged in the face of genomic mutations is considered mutationally robust. However, scientists have debated whether mutational robustness spurs or suppresses adaptation over the long term. Although a broader array of genotypes can be tolerated in a mutationally robust phenotype, the mutations form a "neutral network" that might serve as a genetic resource should the population be confronted with a new environment. Shielding cryptic mutations from G×E interactions in the organism's original environment can allow them to accumulate (see the figure). Theoretical analysis has revealed that mutational robustness can either speed or slow adaptation, depending on whether high-fitness mutations are rare or common, respectively, across the neutral network (2).

Until recently, there have been few experimental tests of whether or how cryptic mutations function in the adaptation of populations. Zheng et al. provide an empirical test to ask whether generating a broad pool of cryptic genetic variations accelerates and diversifies adaptive outcomes when that population requires a specific protein to acquire a new activity. The authors examined yellow fluorescent protein (YFP) function in living *Escherichia coli* cells by using fluorescence-activated cell sorting (FACS) to select cells that display yellow fluorescence in vivo. To generate cryptic genetic variation, they mutagenized the *yfp* gene, introduced the resulting pool into *E. coli* cells, and then selected for the 20% of cells with yellow fluorescence levels that mirrored most closely that of the ancestral phenotype. After four rounds of selection, they created a new selective environment by switching the FACS to select for a green fluorescence—ancestral YFP is weakly fluorescent at the green wavelength—and carried the cells through four rounds of selection for the top 0.1% of YFP variants with the highest green fluorescence activity.

The generation of cryptic variation in YFP before selection for green fluorescence increased the rate of adaptation compared to control populations initiated without prio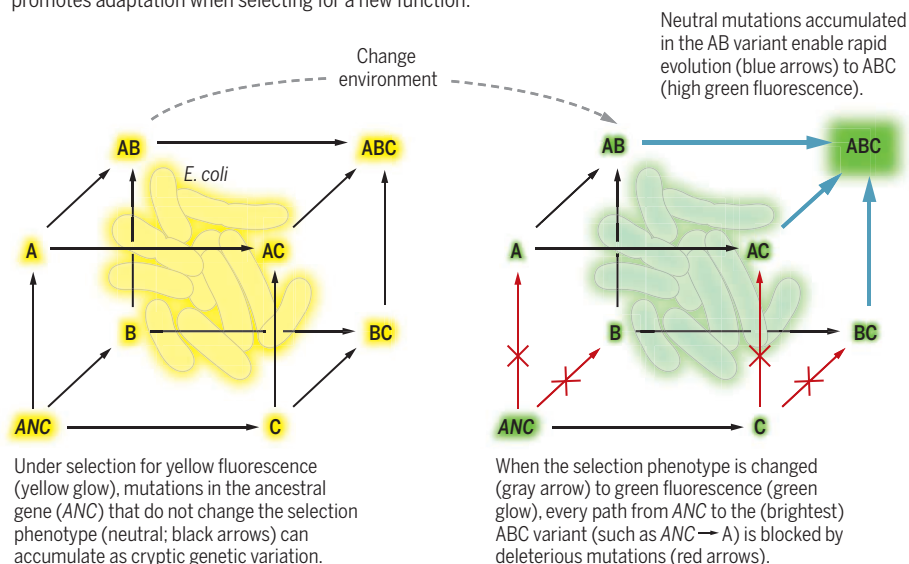r generation of diversity. The benefit of cryptic variation was most prominent in the first round after the transition to selection for green fluorescence. This stands in contrast to results from the in vitro evolution of a ribozyme selected for its ability to use a new substrate, in which boosts in adaptation continued through five rounds of selection (3). Thus, the quantitative effect of cryptic genetic variation is likely to be different across systems and selective pressures.

Zheng et al. also found that the cryptic genetic diversity generated in the first environment permitted evolutionary trajectories that would not otherwise have been accessible. This crucial finding was uncovered by reconstructing all possible mutational intermediates that preceded the end combination, a process that culminated in *yfp* genes that expressed high green fluorescence in the final *E. coli* populations. When green fluorescence was directly selected from the ancestral *yfp* without cryptic variation, the network of mutational intermediates almost exclusively featured steps that, in any order, would have created cells that survive the selection process ("beneficial" mutations). This observation, along with

> *"Zheng et al. greatly advance understanding of how cryptic variation... can aid in adaptation."*

Cryptic mutations facilitate adaptation

Accumulation of mutations that yield neutral changes in a protein promotes adaptation when selecting for a new function.

Under selection for yellow fluorescence (yellow glow), mutations in the ancestral gene (ANC) that do not change the selection phenotype (neutral; black arrows) can accumulate as cryptic genetic variation.

When the selection phenotype is changed (gray arrow) to green fluorescence (green glow), every path from ANC to the (brightest) ABC variant (such as ANC → A) is blocked by deleterious mutations (red arrows).

Neutral mutations accumulated in the AB variant enable rapid evolution (blue arrows) to ABC (high green fluorescence).

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the fact that these populations all ended up with very similar genotypes, demonstrated a constraint on selection. By contrast, nearly all trajectories observed from the pool with cryptic variation featured steps that would have been deleterious in the environment selecting for green fluorescence and, therefore, would not have survived without the initial generation of diversity (see the figure).

Fluorescent proteins and ribozymes manipulated under laboratory conditions represent excellent model systems; but does evidence exist to show that cryptic genetic variation has contributed to the evolution of new traits throughout Earth's history? The answer appears to be yes. An analysis of reconstructed evolutionary intermediates for a family of hormone receptors revealed mutations in the genes that encode these proteins that did not change activity on their own, but were essential for the evolution of new hormone-binding properties more than 400 million years ago (4). A clever genetic screen even allowed researchers to attempt to "replay the tape" to determine the number of possible mutations that would have set the stage for novelty to arise without disrupting the current protein function. They found only one amino acid change that fit these criteria, and it was the one known to have occurred historically (5).

By demonstrating the role of epistasis and the avoidance of G×E interactions through the change of selective conditions, Zheng et al. greatly advance understanding of how cryptic variation—phenotypes that are mutationally robust—can aid in adaptation. The authors suggest that future efforts to use directed evolution for practical purposes incorporate these principles, as is already being done when considering the folding stability and directed evolution of proteins (6). From a fundamental perspective, perhaps the most important question is whether the observations from evolving single RNA or protein molecules also apply at the level of the whole cell; if so, we can expect to move toward a predictive understanding of these phenomena. ∎

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METABOLISM

Lowering ceramides to overcome diabetes

Lowering toxic lipid concentrations in mice has a promising impact on obesity-associated metabolic disorder

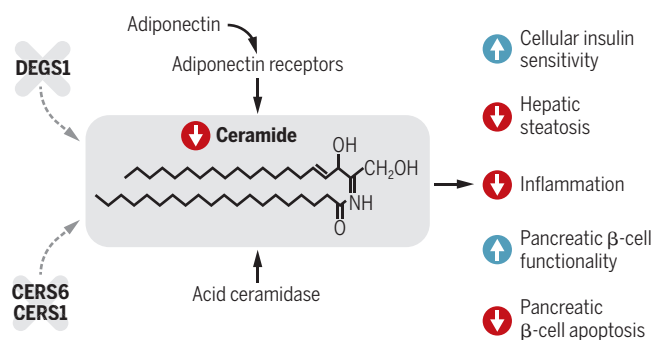By **Christine M. Kusminski** and **Philipp E. Scherer**

Excess nutrient intake leads to a disruption in metabolic homeostasis. In particular, prolonged periods of excess glucose intake can directly contribute to deterioration of insulin sensitivity. Insulin is a key player in the disposal of carbohydrates from food. Frequently associated with this insulin resistance is a dysregulation of lipid metabolism that can lead to lipotoxicity, whereby excess lipids wreak havoc on important intracellular signaling pathways. However, it is largely unknown what types of lipids trigger these cytotoxic effects, which result in further deterioration of glucose and lipid homeostasis. Concentrations of ceramide lipids in blood plasma and tissues are strongly associated with the risk of developing type 2 diabetes (T2D), hepatic steatosis, and cardiovascular disease, which are caused by lipotoxicity and insulin resistance (1, 2). On page 386 of this issue, Chaurasia et al. (3) provide evidence that therapeutically intervening in the ceramide biosynthesis pathway in mice can improve metabolic homeostasis.

The authors focused on a critical, rate-limiting step in the ceramide biosynthesis pathway. Dihydroceramide desaturase 1 (DEGS1) is an enzyme that inserts a double bond into dihydroceramide to produce ceramide. The authors showed that the removal of DEGS1 in mice causes an increase in ceramides lacking a double bond in tissues and plasma. Deletion of the *Degs1* gene in adult mice did not elicit any deleterious effects (an important consideration for possible therapeutic targets, the inhibition of which may lead to unwanted side effects). Furthermore, complete or partial loss of DEGS1 activity substantially improved glucose and lipid metabolism in mice exposed to a high-fat diet.

Chaurasia et al. showed that inhibiting ceramide synthesis in adipocytes or hepatocytes leads to a system-wide improvement in metabolic parameters. This was also observed for components of the ceramide catabolism pathway, such as cell

Intervening in ceramide biosynthesis

In mice, removing dihydroceramide desaturase 1 (DEGS1) or the ceramide synthases CERS1 and CERS6 reduces the amount of available ceramide, which improves metabolic homeostasis.

type-specific overexpression of acid ceramidase, in addition to overexpression of adiponectin receptors (which also have ceramidase activity) (4, 5). Together, this reveals a strong exchange of ceramides throughout the body, such that depleting these lipid species in either hepatocytes or adipocytes lowers ceramides across many tissues involved in maintaining metabolic homeostasis. Adiponectin is a circulating adipokine that signals through its cognate receptors to enhance insulin sensitivity by reducing intracellular ceramides (6). Many clinical studies demonstrate inverse correlations between the amounts of ceramides in plasma and adiponectin in healthy individuals or those with T2D (7–9) (see the figure).

Chaurasia et al. further implicate ceramides in a phenomenon called selective

Science

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