GENETICS

SNPs, Silent But Not Invisible

Single nucleotide polymorphisms that are considered "silent" can affect protein folding.

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Single nucleotide polymorphisms (SNPs) are small genetic changes found in both coding and noncoding regions of the genome. The genetic code is degenerate in that most amino acids are represented by more than one triplet of nucleotide bases (a codon). Such codons are considered synonymous. Many SNPs are "silent" as they result in synonymous codon substitutions. Because these silent SNPs do not change the amino acid composition of the protein product, they have largely been assumed to exert no discernible effect on gene function or phenotype. But are silent SNPs indeed always silent? On page 525 in this issue, Kimchi-Sarfaty et al. (1) provide evidence that naturally occurring silent SNPs can affect in vivo protein folding and, consequently, function. The study shows that substrate specificity of P-glycoprotein, the product of the multidrug resistance 1 (MDR1) gene, is altered by SNPs presumed to be synonymous and silent. How is this possible?

The original Beadle and Tatum hypothesis of "one gene, one enzyme"—the essence of the classic view of gene structure and function—started to break down almost as soon as it had been completely formulated (2). Cellular processes, including alternative RNA splicing and posttranslational protein modifications, create more than one protein product from a given sequence in the genome. Yet, it is widely assumed that a single messenger RNA (mRNA) encoding a polypeptide chain with a defined amino acid sequence will give rise to, during the process of translation, identical protein products in terms of their structure and activity, assuming that these products undergo the same co- and posttranslational modifications in the cell. This assumption is based on the widely accepted Anfinsen's principle, which states that the information necessary to specify the native three-dimensional structure of a protein—which largely defines its function—is contained solely in its amino acid sequence (3).

Although these two principles can still be applied to a wealth of genes and proteins, a polypeptide chain can exist in the cell in a number of alternative conformations, revealing different functions and/or activities. For

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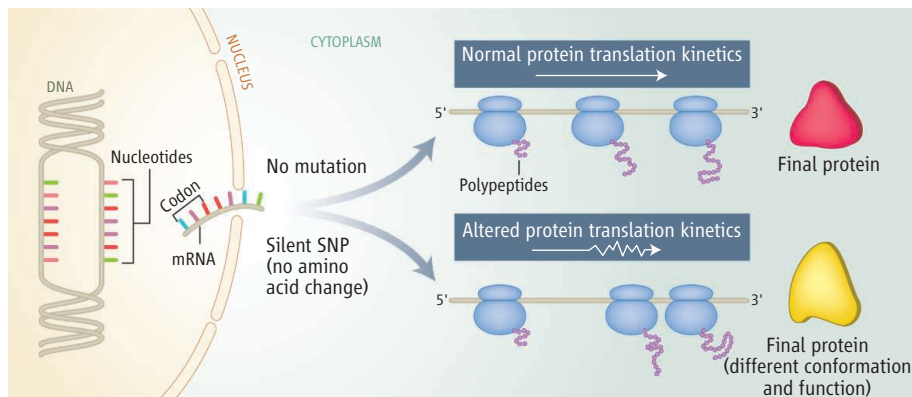
Translation kinetics and protein folding. Unaffected translation kinetics results in a correctly folded protein. Abnormal translation kinetics, caused by the ribosome moving faster or slower through certain mRNA regions, can produce a different final protein conformation. Abnormal kinetics may arise from a silent single nucleotide polymorphism (SNP) in a gene that creates a codon synonymous to the wild-type codon. However, this synonymous codon substitution may lead to different kinetics of mRNA (protein) translation, thus yielding a protein with a different final structure and function.

example, a neuronal isoform of cytoplasmic polyadenylation element binding protein in the sea slug (Aplysia californica) exists in two different forms: a soluble inactive form and an insoluble active form that regulates synaptic protein synthesis (4). The molecular mechanisms that can drive the folding of a polypeptide chain in vivo into alternative conformations are still mostly obscure.

The crowded cellular environment, folding catalysts, and accessory proteins all contribute to protein folding in the cell (5). Could there be other factors affecting the final conformation of a protein? In 1987, Purvis and colleagues proposed that "the way in which some proteins fold is affected by the rates at which regions of their polypeptide chains are translated in vivo" (6). They hypothesized that certain "gene sequences have evolved to control the rate of translational elongation such that the synthesis of defined portions of their polypeptide chains is separated temporally."

The rate of ribosome traffic on mRNA is known to be nonuniform and mainly modulated by both the general degeneracy of the genetic code (namely, the use of specific synonymous codons that is characteristic for a given organism) and the availability of cognate aminoacyl–transfer RNAs (tRNAs) that surround the ribosome during translation, poised to deliver amino acids. Within many organisms, a strong codon bias exists (7) and the amount of cognate tRNAs is directly proportional to the frequency of codon usage.

Thus, infrequent codons in mRNA appear to be slowly translated, whereas frequent codons are rapidly translated.

Optimization of in vivo protein folding—which is thought to be cotranslational (5)—might include the adaptation of codon selection along DNA (and hence, along corresponding mRNA) to a particular translation kinetics. Temporal separation of folding events during protein synthesis on the ribosome might thus avoid "unwanted" interactions within the growing polypeptide, ensuring high yield of the correctly folded protein. Therefore, altered translation kinetics of a defined mRNA due to, in particular, synonymous codon substitutions (which would not alter the amino acid sequence of the encoded polypeptide) might drive the in vivo folding of the same polypeptide chain into different conformations (see the figure).

This hypothesis is very difficult to prove using in vivo systems, because numerous quality-control mechanisms exist to get rid of incorrectly or abnormally folded and misfolded proteins (8). So far, supporting experimental data have been scarce, making the findings of Kimchi-Sarfaty et al. particularly important. The authors show that combinations of the three previously known SNPs for the MDR1 gene (C1236T, G2677T, and C3435T) alter P-glycoprotein activity. P-glycoprotein is a multiple-transmembrane protein pump that transports various drugs out of cells. Its function can thus reduce the efficacy

of chemotherapy treatments. Kimchi-Sarfaty *et al.* found that P-glycoprotein inhibitors CsA and verapamil were less effective against proteins that were produced from haplotypes that consist of the polymorphic double (C1236T-G2677T, C1236T-C3435T, G2677T-C3435T) or triple (C1236T-G2677T-C3435T) variant combinations, suggesting that these protein products have altered conformations. Yet, C1236T and C3435T polymorphisms do not change the amino acid sequence of P-glycoprotein. The C1236T polymorphism changes a GGC codon to GGT at amino acid position 412 of the polypeptide (both encode glycine) and the C3435T polymorphism changes ATC to ATT at position 1145 (both encode isoleucine). However, both polymorphisms result in changes from frequent to infrequent codons and therefore may slow down the ribosome traffic at the corresponding mRNA regions. These alterations may thus affect the cotranslational folding pathway of P-glycoprotein, resulting in a different final confor-

mation. Limited proteolysis and the use of a conformation-sensitive monoclonal antibody indeed revealed structural differences between the wild-type protein and the polymorphic haplotypes.

Artificial site-directed silent mutagenesis of synonymous codons (changing from infrequent to frequent) in certain genes also support the hypothesis that altered translation kinetics of mRNA might affect final protein conformation (*9*). However, until the study by Kimchi-Sarfaty *et al.*, there had been no example demonstrating that naturally occurring variations in synonymous codons in a defined gene can give rise to a protein product with the same amino acid sequence but different structural or functional features. By demonstrating that this is indeed the case, the study opens up a new avenue of research and suggests that silent SNPs might contribute to development and progression of certain diseases. If this is the case, then silent SNPs should not be neglected in determining the

likelihood of the development and progression of many diseases such as Alzheimer's disease, myopia (a disease leading to a refractive defect of the eye), and others that are strongly linked to SNPs. This knowledge should also be taken into account in personalized drug treatment and development programs.

References and Notes
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ATMOSPHERES

The Jet-Stream Conundrum

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Fluid jets occur in Earth's atmosphere and oceans, and on other planets. A new theoretical view of jets has helped reveal why they form and how they interact with the surrounding fluid.

Jet streams, or "jets" for brevity, are concentrated, intense, elongated flows that often contain most of the kinetic energy in a flowing fluid. They are pervasive features of Earth's atmosphere and oceans, where they transport heat, chemicals, and even biota such as krill, and they are also abundant on other planets (see the figure).

Jets are observed to occur spontaneously on rotating planets whenever stratified atmospheres or oceans are forced into turbulent motion. Yet there is a mystery as to why jets exist at all—why is there this propensity to concentrate energy and momentum? A second mysterious property of jets is that they can act as flexible material barriers, inhibiting mixing across their axes. The strongest eastward jets provide expressways for the transport of chemicals and biota along their axes but severely inhibit mixing across their axes. A

new theoretical paradigm (*1*) explains the abundance of jets, in any planetary atmosphere or ocean, in a simple manner. The paradigm combines field theory with chaotic (turbulent) fluid motion; in doing so, it captures long-range interactions that are crucial for forming and stabilizing jets. It shows that mix-

ing a fluid on a rotating planet will invariably produce jets and that the two mysteries, jet formation and the inhibition of mixing, formerly regarded as two separate phenomena, are intimately related to each other.

Both jet formation and the inhibition of mixing are completely enigmatic in terms of

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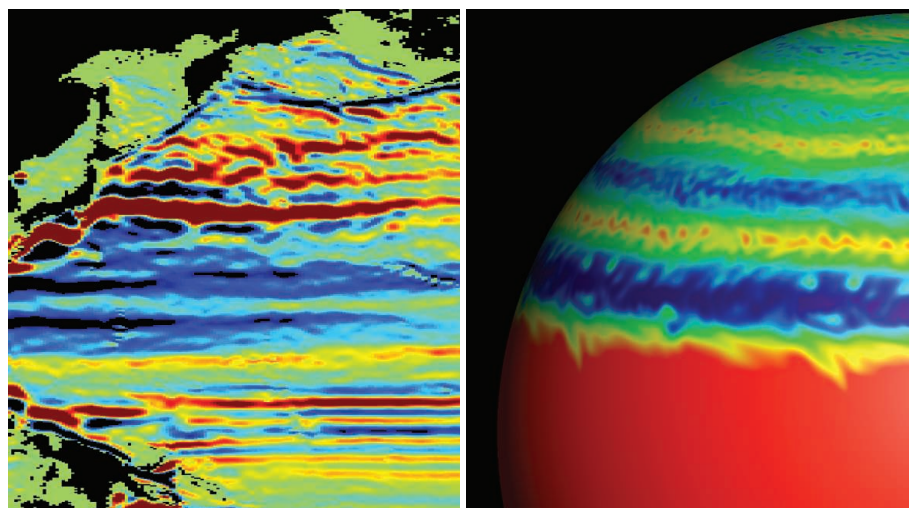
Jets near and far. (Left) Map of east-west current speeds at 400-m depth, simulated by an eddy-resolving ocean model. Red and blue indicate eastward and westward flows. [Adapted from Richards *et al.* (*11*)] **(Right)** Snapshot of a simulation for Jupiter, with red and blue indicating eastward and westward flows. [Adapted from Heimpel *et al.* (*16*)]