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Non-equilibrium coupling of protein structure and function to translation–elongation kinetics

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Protein folding research has been dominated by the assumption that thermodynamics determines protein structure and function. And that when the folding process is compromised *in vivo* the proteostasis machinery — chaperones, deaggregases, the proteasome — work to restore proteins to their soluble, functional form or degrade them to maintain the cellular pool of proteins in a quasi-equilibrium state. During the past decade, however, more and more proteins have been identified for which altering only their speed of synthesis alters their structure and function, the efficiency of the down-stream processes they take part in, and cellular phenotype. Indeed, evidence has emerged that evolutionary selection pressures have encoded translation-rate information into mRNA molecules to coordinate diverse co-translational processes. Thus, non-equilibrium physics can play a fundamental role in influencing nascent protein behavior, mRNA sequence evolution, and disease. Here, we discuss how our understanding of this phenomenon is being advanced by the application of theoretical tools from the physical sciences.

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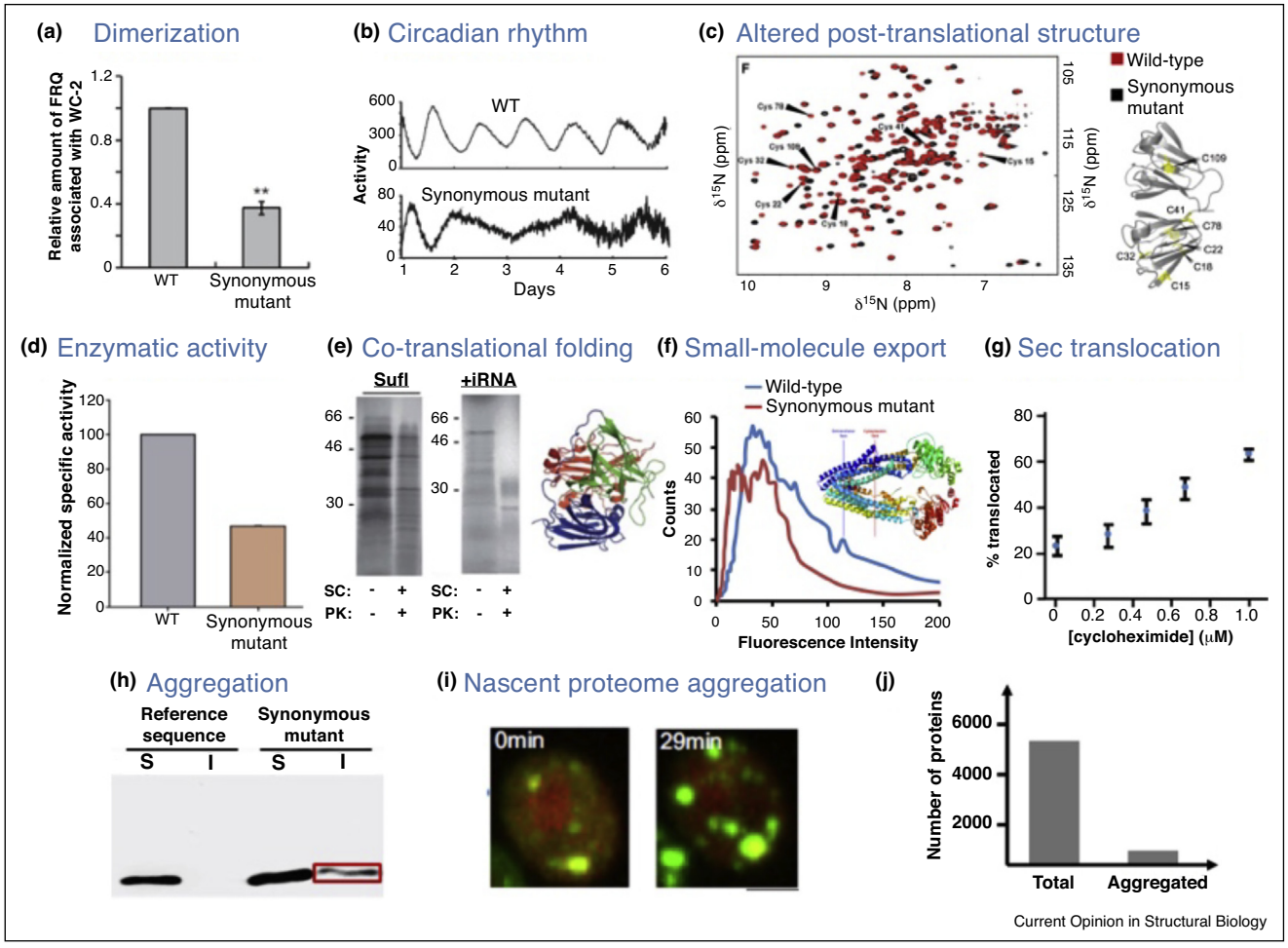
Introduction

A large body of evidence now demonstrates that the structure and function of some newly synthesized proteins is influenced by the rate at which individual codon positions in an mRNA molecule are translated by the ribosome during protein synthesis. This phenomenon was discovered by introducing synonymous codon mutations into transcripts that altered the rates of translation while leaving the encoded amino acid sequence unaltered. Such variation in codon translation rates has been found to influence whether a nascent protein will fold and function [1–4], misfold and malfunction [5–9], aggregate [10,11], or efficiently translocate to a different cellular compartment [12] (Figure 1). As a consequence, downstream cellular processes are also perturbed, including the ability of these proteins to correctly dimerize [13], catalyze reactions [3], import and export material into or out of the cell [8], and maintain circadian rhythm [13]. Thus, synonymous codon substitutions and their attendant changes in translation–elongation rates can govern the fate of nascent proteins in cells and can drastically affect cellular processes and organismal fitness [14,15]. Indeed, a number of human diseases have recently been linked to synonymous codon mutations that can change translation–elongation rates [16••,17,18]. These diseases include particular subtypes of hemophilia [19], lung carcinoma, and cervical and vulvar cancers [16••,17]. There are likely to be many more diseases caused by synonymous codon mutations that have not yet been discovered, since the research community has long assumed such mutations do not affect an organism's phenotype.

These observations necessitate a shift, or broadening [9], in perspective from Anfinsen's Dogma of thermodynamic control of protein structure and function towards a non-equilibrium perspective in which translation kinetics, in conjunction with thermodynamic driving forces for self-assembly, can play a significant role in the gain or loss of function of nascent proteins *in vivo*. To be sure, cells, and hence proteins in those cells, exist in an out-of-equilibrium environment. Nonetheless, it is commonly assumed that many cellular processes occur on time scales much longer than that of protein folding, leading to the effective equilibration (i.e., thermodynamic control) of a protein during its lifetime in a cell. If the thermodynamic perspective is the primary determinant of nascent protein behavior then the impact of translation kinetics should be quickly 'forgotten' by the protein molecule [14] and have no impact on its long-time scale behavior *in vivo*. It is exactly this prediction that studies have shown not to be true for a variety of proteins [1,3,8,13].

Recently, application of tools from the physical sciences that describe the time evolution of out-of-equilibrium systems have improved our ability to understand, model and predict the influence of translation kinetics on nascent protein behavior. These tools include molecular

Figure 1



Evidence that translation–elongation kinetics influence nascent protein behavior and down-stream cellular processes. **(a)** Faster codon translation rates for the synonymously mutated construct of FRQ decreases its ability to bind protein WC-2 by 60% in comparison to wild-type and **(b)** abolishes the circadian rhythm of *Neurospora* [13]. **(c)** Differences in codon translation rates between the wild-type and synonymously mutated γB-crystallin transcripts lead to altered disulfide bond formation and different folded conformations as determined by NMR [53•]. **(d)** Synonymously mutating firefly luciferase's transcript to translate more quickly reduces its specific activity by ~50% in comparison to the wild-type transcript's product [54]. **(e)** A three-domain SufI protein folds co-translationally (lanes marked SufI). Increasing the concentration of tRNA increases codon translation rates (lanes marked +tRNA), reducing the ability of SufI to fold during translation and decreasing its resistance to proteinase K (PK) digestion. SC denotes sucrose cushion separation [1]. **(f)** The introduction of three synonymous mutations to the MDR1 gene results in a significant change to the drug export functionality of the MDR1 gene product, P-glycoprotein, in comparison to the wild-type [8]. Cancer patients with these mutations do not respond to treatment due to the altered drug export functionality of P-glycoprotein. The intracellular accumulation of the fluorescent substrates measures the functionality of P-glycoprotein. **(g)** Cycloheximide globally decreases codon translation rates, drastically increasing the amount of pre-prolactin that is SEC translocated [12]. **(h)** Introducing three fast-translating synonymous mutations at a key location between domains (synonymous mutant) into the *Echinococcus granulosus* fatty-acid binding protein 1 dramatically increases the amount of insoluble [I], aggregated protein, indicating that synonymous mutations are promoting the protein's misfolding [11]. **(i)** Introduction of the non-natural amino acid A2C, which is known to induce misfolding, causes new aggregates to form in yeast cells, indicating nascent protein misfolding can drive aggregation [80]. **(j)** Mass spectrometry revealed that the introduction of a tRNA modification that globally altered translation speed led to an increase in the aggregation of 610 *S. cerevisiae* proteins out of the total 5400 proteins [81•]. Figure a and b are reprinted by permission from Macmillan Publishers Ltd: Nature (Ref. [13]), copyright (2013). Figure c is reprinted from Molecular Cell, 61, Buhr et al., synonymous codons direct cotranslational folding toward different protein conformations, 341, copyright (2016), with permission from Elsevier. Figure d is reprinted from Journal of Molecular Biology, 422, Spencer et al., silent substitutions predictably alter translation elongation rates and protein folding efficiencies, 328, copyright (2012), with permission from Elsevier. Figure e is reprinted by permission from Macmillan Publishers Ltd: Nature Structural and Molecular Biology (Ref. [1]), copyright (2009). Figure h is reprinted from Biochemical and Biophysical Research Communications, 293, Cortazzo et al., silent mutations affect in vivo protein folding in *Escherichia coli*, 537, copyright (2002), with permission from Elsevier. Figure i is reprinted from Cell, 159, Zhou et al., organelle-based aggregation and retention of damaged proteins in asymmetrically dividing cells, 530, copyright (2014), with permission from Elsevier.