Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Repurposing the translation apparatus for synthetic biology

Benjamin J Des Soye[1,2,3,4,8], Jaymin R Patel[5,6,8], Farren J Isaacs[5,6] and Michael C Jewett[1,2,3,4,7]

The translation system (the ribosome and associated factors) is the cell's factory for protein synthesis. The extraordinary catalytic capacity of the protein synthesis machinery has driven extensive efforts to harness it for novel functions. For example, pioneering efforts have demonstrated that it is possible to genetically encode more than the 20 natural amino acids and that this encoding can be a powerful tool to expand the chemical diversity of proteins. Here, we discuss recent advances in efforts to expand the chemistry of living systems, highlighting improvements to the molecular machinery and genomically recoded organisms, applications of cell-free systems, and extensions of these efforts to include eukaryotic systems. The transformative potential of repurposing the translation apparatus has emerged as one of the defining opportunities at the interface of chemical and synthetic biology.

Addresses
[1] Interdisciplinary Biological Sciences Program, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA
[2] Northwestern Institute on Complex Systems, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA
[3] Simpson Querrey Institute, Northwestern University, 303 East Superior Street, Suite 11-131, Chicago, IL 60611, USA
[4] Chemistry of Life Processes Institute, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA
[5] Systems Biology Institute, Yale University, West Haven, CT 06516, USA
[6] Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06510, USA
[7] Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA

Corresponding authors: Isaacs, Farren J (farren.isaacs@yale.edu) and Jewett, Michael C (m-jewett@northwestern.edu)

[8] These authors contributed equally to this work.

Current Opinion in Chemical Biology 2015, **28**:83–90

This review comes from a themed issue on **Synthetic biology**

Edited by **Michelle Chang** and **Huimin Zhao**

http://dx.doi.org/10.1016/j.cbpa.2015.06.008

Background

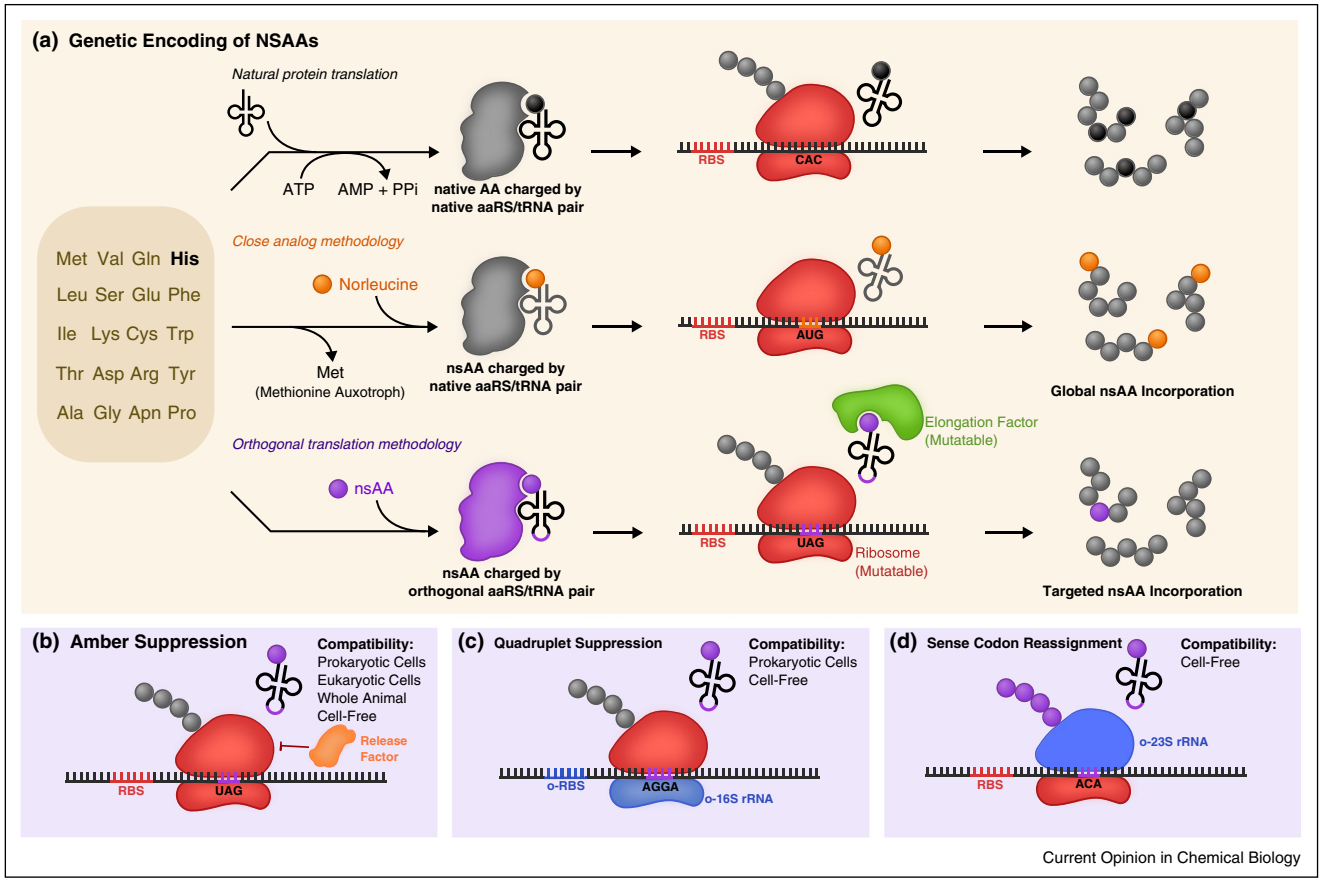
Proteins represent a crucial class of biomolecules, universally employed by *all* living organisms to fulfill essential structural, functional, and enzymatic roles necessary to support life. In nature, these polymers are composed generally of twenty natural amino acid (AA) building blocks, which can be combined in a near-infinite number of combinations to generate an impressive level of structural and functional diversity (Figure 1). However, many interesting chemistries cannot be accessed using only these natural building blocks; accordingly, for some time there has been an interest in the incorporation of non-standard amino acids (nsAAs) featuring novel functional sidegroups to expand the repertoire of protein functions.

Broadly speaking, nsAAs can be divided into two classes. *Synthetic nsAAs* are chemically synthesized, and can bear little resemblance to their naturally occurring counterparts. *Posttranslational modifications* (PTMs) are modified derivatives of canonical amino acids. In recent years, two distinct approaches for the incorporation of nsAAs into proteins have emerged (Figure 1(a)). One such approach is *global suppression*. This method uses auxotrophic strains that are incapable of synthesizing a particular AA. When grown in the presence of a nsAA that bears close structural resemblance to the 'missing' AA, the organism's native translational machinery incorporates the nsAA instead [1,2]. An alternative approach uses orthogonal translation systems (OTSs) to genetically encode an nsAA of interest site-specifically by reassignment of codons, typically the amber stop codon (TAG) in a strategy known as *amber suppression* [3].

To date, >150 nsAAs have been incorporated by OTSs into polypeptides [4] for a wide range of applications including the introduction of bioorthogonal handles for protein tagging [5,6], alteration of protein stability [7,8], monitoring of protein localization, and genetic encoding of PTMs [9•,10–12]. As a result of these impressive efforts and the transformative potential to construct bio-based products beyond natural limits, expanding the genetic code has emerged as one of several major defining opportunities and points of synergy in chemical and synthetic biology.

This review focuses on recent developments in repurposing the translation system for novel functions, with a focus on codon reassignment. We first examine development of the molecular machinery at the heart of genetic code expansion. Next, we discuss nsAA incorporation in several contexts, including whole-genome recoding, prokaryotic and eukaryotic systems *in vivo* as well as *in vitro*. We

Figure 1



Methods for genetic code expansion. **(a)** Two general paradigms exist for the genetic incorporation of nonstandard amino acids into proteins contrasted with the natural process of encoding the canonical amino acids. The close analog methodology complements a natural amino acid auxotrophy with a close nonstandard analog, enabling global protein labeling by native translational machinery. The orthogonal translation methodology introduces orthogonal translational machinery engineered to charge an orthogonal tRNA with a nonstandard amino acid, enabling site-specific targeted genetic incorporation. Certain nsAAs may require additional mutations in the elongation factor or the ribosome. **(b)** For targeted genetic incorporation, amber suppression is the most widely used technique. Competition with release factors limits efficiency, and methods are discussed to overcome this. **(c)** Quadruplet suppression can be performed with appreciable efficiency with the use of an engineered orthogonal 16S ribosomal subunit [27••]. **(d)** Sense codons can be reassigned by using an orthogonal 23S ribosomal subunit, engineered to accept a synthetic set of tRNAs [60].

end with a discussion of current challenges in the field and provide commentary on future opportunities.

Genetic code expansion using OTSs

Amber suppression seeks to 'hijack' the amber translational stop codon (TAG), recoding it into a sense codon corresponding to a nsAA of interest. Generally, this is accomplished using a *suppressor tRNA* that has been mutated to decode the amber codon and an *aminoacyl-tRNA synthetase* (aaRS) that has been mutated to accept the nsAA of interest and covalently load it onto the suppressor tRNA. These components are typically sourced from distant archeal species to ensure orthogonality to host translation machinery, undergoing directed evolution to improve their compatibility with a new nsAA and enable its site-specific incorporation into proteins.

Directed evolution is the most widely used approach for the generation of novel OTS components [13–15] (Figure 2). These efforts start with the selection of a scaffold aaRS/tRNA pair. To date, several aaRS/tRNA pairs have been used in the creation of new OTSs. The *Methanocaldococcus jannaschii* TyrRS/tRNA^{Tyr} pair is arguably the most common pair used, but is generally limited to aromatic amino acids and is not orthogonal in eukaryotes [4,15,16•]. The PylRS/tRNA^{Pyl} pair from *Methanosarcina* species (*M. mazei*, *M. barkeri*) has shown compatibility with eukaryotic systems [16•,17], and is an especially attractive starting point for evolution as the native PylRS natively demonstrates polysubstrate specificity [18] and tRNA^{Pyl} natively decodes the amber codon [19]. Other starting components have included the *o*-phosphoserine (Sep)RS from *Methanococcus maripaludis*