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Current Opinion in Chemical Biology

Synthesis at the interface of virology and genetic code expansion

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How a virus efficiently invades its host cell and masterfully engineers its properties provides valuable lessons and resources for the emerging discipline of synthetic biology, which seeks to create engineered biological systems with novel functions. Recently, the toolbox of synthetic biology has also been enriched by the genetic code expansion technology, which has provided access to a large assortment of unnatural amino acids with novel chemical functionalities that can be site-specifically incorporated into proteins in living cells. The synergistic interplay of these two disciplines holds much promise to advance their individual progress, while creating new paradigms for synthetic biology. In this review we seek to provide an account of the recent advances at the interface of these two research areas.

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Current Opinion in Chemical Biology 2018, **46**:164–171

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

Edited by **Michael Ledbetter** and **Floyd E Romesberg**

https://doi.org/10.1016/j.cbpa.2018.07.015

Introduction

Viruses employ highly sophisticated strategies to infect host cells with the ultimate goal of using the host machinery and resources for their self-replication. The virus capsid, as well as the proteins and RNAs encoded in the viral genome, have evolved to possess numerous fine-tuned functions to facilitate various stages of this process including viral entry, altering the physiology of the host cell for optimal viral replication, packaging of progeny virus, and their escape back to the environment. In addition to their importance in fundamental virology and the development of new antiviral therapeutics, understanding the molecular processes associated with viral infection can provide important lessons and resources to further enrich the toolbox for synthetic biology. Indeed, genetic elements derived from viruses (e.g., promoters, nucleic acid polymerases, internal ribosome-entry site elements, and so on) are frequently borrowed in numerous synthetic biology applications. Additionally, the efficient cellular entry process of different viruses provides an attractive roadmap to create next-generation delivery vectors for a variety of therapeutic agents, including 'gene circuits'.

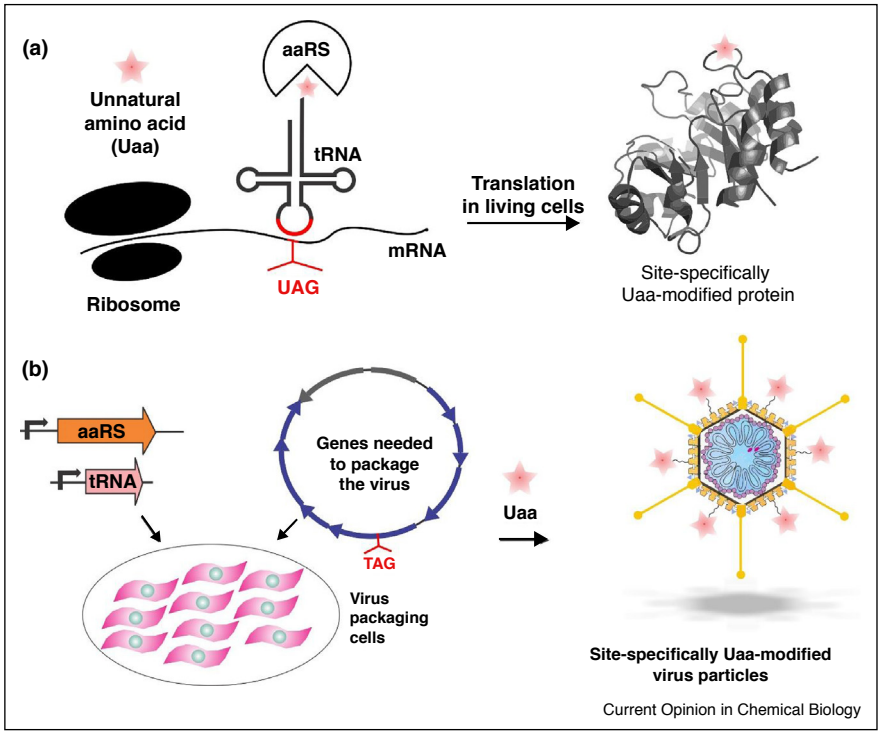
The genetic code expansion (GCE) technology enables site-specific incorporation of unnatural amino acids (Uaas) into proteins expressed in living cells, using engineered aminoacyl-tRNA synthetase (aaRS)/tRNA pairs that suppress a repurposed nonsense codon (Figure 1a) [1–3]. This technology has experienced remarkable progress over the last decade, facilitating its application to various domains of life [1–3]. Using this approach, over 150 different Uaas with novel sidechains have been genetically encoded to date, providing an exciting new toolset in the arsenal of synthetic biology.

In recent years, there have been exciting developments at the interface of the GCE technology and virology. It has become increasingly clear that the tools from each of these disciplines are particularly well-suited to expand the scope of the other. In this review we attempt to capture the relevant body of work demonstrating how this synergistic interplay is accelerating the progress of these two disciplines and creating new opportunities for synthetic biology.

Chemical modification of virus-like particles (VLPs)

Virus-like particles (VLPs) are protein cages resembling viruses, assembled from capsid proteins of viruses (e.g., MS2, Qβ, TMV) or other proteins [4,5]. Despite lacking the highly sophisticated properties of live viruses for efficient cell-entry, they have been developed as vehicles to deliver small-molecule cargo *in vitro* and *in vivo*, including imaging agents and cytotoxic drugs, as well as for vaccine development [6,7•,8,9]. Since several excellent reviews have already been published on this topic, here we briefly summarize such efforts that take advantage the GCE technology. Chemical modification has been widely used to functionalize VLPs with both targeting agents and cargo [10–12]. However, functionalizing canonical amino acid residues on VLPs intrinsically lacks site-specificity, unless a restricted number of target residues are surface-accessible. The GCE technology provides an attractive alternative by enabling site-specific incorporation of Uaas, which can be subsequently

Figure 1



(a) A general scheme demonstrating the site-specific incorporation of Uaas into proteins expressed in living cells using a nonsense suppressing engineered aaRS/tRNA pair. **(b)** Production of virus particles site-specifically labeled with Uaas.

functionalized using chemoselective labeling strategies. Finn, Tirrell *et al.* used a global methionine replacement strategy to introduce azidohomoalanine (Aha) and homopropargylglycine (Hpg) into the coat protein of Qβ derived VLPs on both the inner and outer surfaces, which were later functionalized with fluorophores, cell-targeting glycans, and so on, through azide-alkyne click chemistry (Figure 2b; Table 1) [10,13]. VLPs have also been generated incorporating Aha or Hpg using a bacterial cell-free protein expression system and subsequently functionalized with PEG, nucleic acids, and proteins [14]. Taking advantage of a previously developed engineered aaRS/tRNA pair that charges *p*-aminophenylalanine (pAmF) in response to the UAG nonsense codon [15], the Francis group has site-specifically introduced this Uaa onto the surface of Qβ VLPs. Using its chemoselective oxidative coupling with electron-rich aromatic amines (Figure 2b, Table 1), the pAmF residues were subsequently labeled with PEG [16] and cell-targeting groups including peptides [17], aptamers [18], proteins [19], and antibodies [20]. This labeling strategy has been further combined with cysteine functionalization on the interior of the capsid to deliver PET [21] and MRI [22] imaging agents and cytotoxic photodynamic therapy agents [23] to cancer cells *in vivo*, and for *in vivo* imaging of fibrin clots [24]. Plant-derived tobacco mosaic virus (TMV) VLPs, which assemble into rod or disc shapes depending on conditions, have also been functionalized with Uaas using the GCE technology [25].

Using genetic code expansion to probe and engineer viruses

The ability to selectively modify the proteins associated with viruses is important both for understanding their roles in viral replication through the attachment of biophysical/biochemical probes, and from the perspective of engineering viral vectors with desirable traits (e.g., cell selectivity, immune evasion, and so on). Unlike VLPs, viruses employ a series of complex molecular maneuvers to enter their host cells with remarkable efficiency [26–30]. To ensure that these delicate processes are not perturbed, extra care must be taken when introducing modifications on the virus particle. Indeed, the proteins associated with various mammalian viruses are remarkably complex and multifunctional [26–30]. Consequently, these are often refractory to traditional genetic manipulations, such as incorporation of peptide or protein tags, the large size of which can significantly perturb their native function. In one of the first examples of chemoselective modification of mammalian viruses, the Carrico group generated azide-labeled adenovirus particles through metabolic incorporation of azido-containing carbohydrates as well as global replacement of methionines with azidohomoalanine (Aha) [31,32]. The resulting azide