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Current Opinion in Biotechnology

Amino acid misincorporation in recombinant biopharmaceutical products

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Microbial and mammalian host systems have been used extensively for the production of protein biotherapeutics. Generally these systems rely on the production of a specific gene sequence encoding one therapeutic product. Analysis of these protein products over many years has proven that this was not always the case, with multiple species of the intended product being produced due to amino acid misincorporation or mistranslation during biosynthesis of the protein. This review is the first to give a comprehensive overview of the occurrence and analysis of these misincorporations. Furthermore, using the latest data on misincorporation in native human proteins we explore potential considerations for producing a specification for misincorporation for the development of a human biotherapeutic protein product in a production environment.

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Current Opinion in Biotechnology 2014, **30**:45–50

This review comes from a themed issue on **Pharmaceutical biotechnology**

Edited by **Beth Junker** and **Jamey D Young**

http://dx.doi.org/10.1016/j.copbio.2014.05.003

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Introduction

Advances in the manipulation of DNA in the later part of the last century resulted in the extensive use of recombinant host systems to produce heterologous proteins. Initially this was shown through the use of *Escherichia coli* in the production of Insulin, which was the vanguard for the use of the recombinant host systems that followed. These systems include mammalian cells, yeasts, insect cells and filamentous fungi which have resulted in the commercialisation and approval of now more than 150 recombinant products [1,2]. Bioprocess optimisation and product cost of goods (COGS) are paramount to the success of any chosen production platform for these products [3]. Therefore the maximum product titre that can be achieved is highly desirable. This can produce many challenges for production of complex recombinant biomolecules that are not native to the host organism. Production in this unnatural environment can promote stress or metabolic burden on cells which can result in the production of incorrect or unintended products. This can be magnified further when these protein products are being produced under the stresses of fed-batch or continuous high density cultures, for example. In cases where these factors lead to significantly different proteins (e.g. gene frameshifts or stop codon over runs), it is relatively simple to detect and then remove the unintended population by vector/gene redesign (in the case of rare codons) or via downstream processing (DSP). Amino acid misincorporation however presents an interesting challenge as the resulting protein species can vary by only one amino acid and at a very low level which can be difficult to detect [4]. In the past it was estimated that this translational mismatch occurred on a level of 10^{-5} to 10^{-3} per translated codon [5–8] but increases significantly under stress from the production environment [9•,10–12]. The effect of amino acid misincorporation can be invisible as it occurs at a trace level, and also may have little or no adverse effect on protein activity. This may be a reason that it is not fully understood or well documented (especially with pharmaceutical proteins that have made it to market). Of overriding concern is that these unnatural species of the protein product could have an immunogenic response when destined for the clinic. This concern has been raised previously [4,13,14], but is yet to be fully documented and understood. As mammalian and *E. coli* systems are used extensively in the production of recombinant biopharmaceutical products; the misincorporation of amino acids in these systems will be discussed here in more detail.

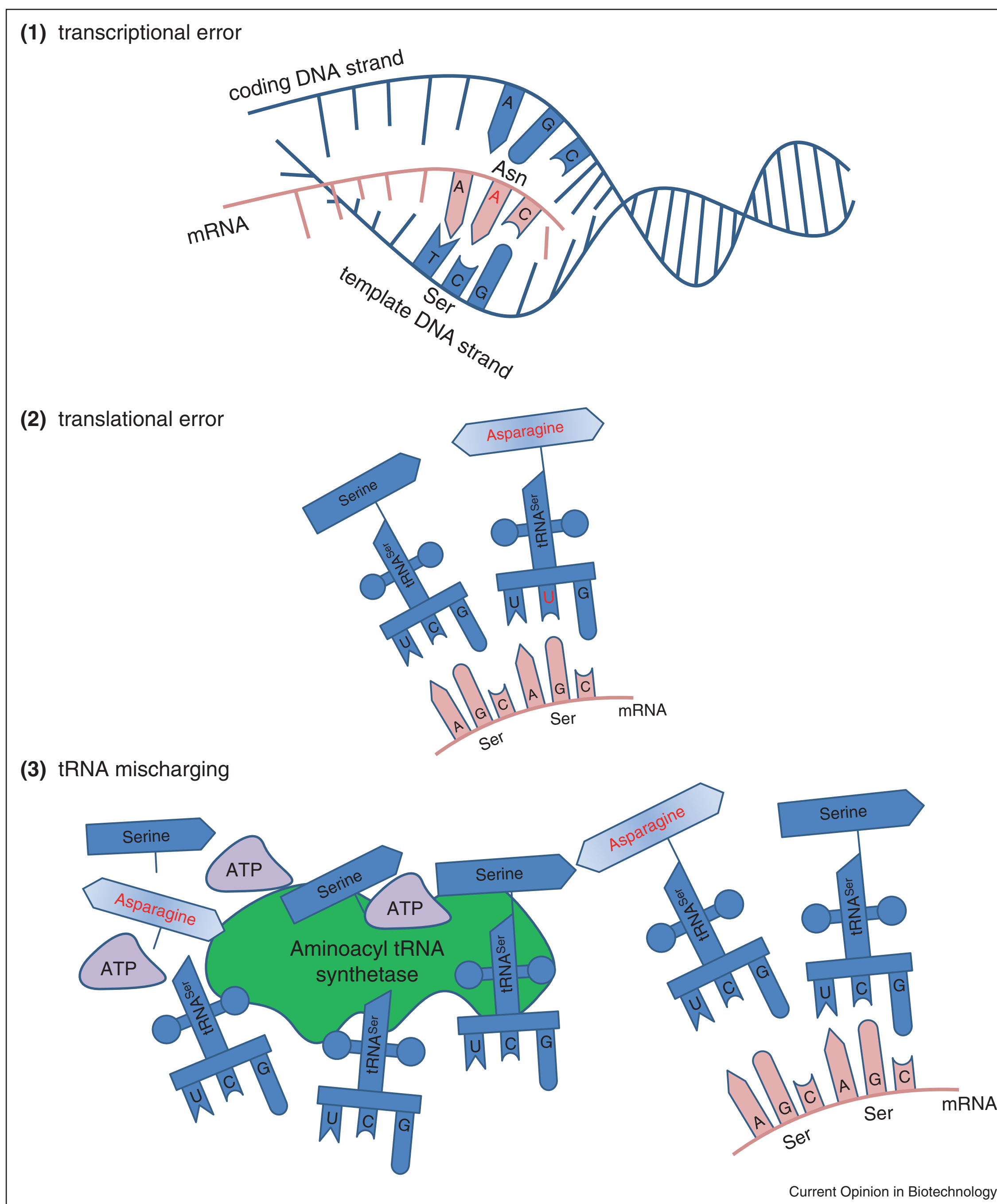
Mechanisms of amino acid misincorporation

Production of recombinant products by the pharmaceutical industry relies on the transcription and translation of a specific gene sequence encoding one therapeutic protein. The synthesis of a protein is as a result of the transfer of the genetic blueprint via RNA species to formation of the polypeptide. During this process of construction there can be a background level of errors which under balanced growth conditions can result in a very low occurrence of error [15].

Three types of error can arise during transcription/translation of a gene to protein: (A) frame shifting, (B) stop codon over-runs and (C) amino acid misincorporation or mistranslation [14]. Because of the nature of the errors in (A) and (B) the protein translated exhibits vastly different

characteristics to the desired product (e.g. different charge or hydrophobicity). In the case of a highly purified biopharmaceutical these proteins can be removed during downstream processing (DSP) with minimal effort. However, the mistranslation error (C) results in a protein species that can vary by only one amino acid. Removal of these populations will be difficult as the species are likely to exhibit similar properties, and initial identification may be missed due to the very low levels of error. Common causes of misincorporation or mistranslation of an amino acid are due to one of the following mechanisms (see Figure 1):

Figure 1



Mechanisms for misincorporation of amino acids. Schematics show three potential routes to formation of an incorrectly encoded amino acid (serine to asparagine) via (1) transcriptional, (2) translational, and (3) tRNA mischarging errors. mRNA editing is also described as a possible cause of misincorporation of amino acids [18••].

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