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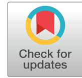
Understanding biopharmaceutical production at single nucleotide resolution using ribosome footprint profiling



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Biopharmaceuticals such as monoclonal antibodies have revolutionised the treatment of a variety of diseases. The production of recombinant therapeutic proteins, however, remains expensive due to the manufacturing complexity of mammalian expression systems and the regulatory burden associated with administrating these medicines to patients in a safe and efficacious manner. In recent years, academic and industrial groups have begun to develop a greater understanding of the biology of host cell lines, such as Chinese hamster ovary (CHO) cells and utilise that information for process development and cell line engineering. In this review, we focus on ribosome footprint profiling (RiboSeq), an exciting next generation sequencing (NGS) method that provides genome-wide information on translation, and discuss how its application can transform our understanding of therapeutic protein production.

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Introduction

Recombinant therapeutic proteins currently represent a considerable portion of the biopharmaceutical industry. The effectiveness of these molecules in treating a variety of diseases including auto-immune conditions and cancer is reflected in annual sales that now exceed $140 billion USD [1]. Patient access to biopharmaceuticals, particularly complex glycoproteins (e.g. monoclonal antibodies (mAbs) and Fc-fusion proteins), can be limited by cost or the inability of expression hosts, such as Chinese hamster ovary (CHO) cells, to synthesise the protein in economically viable quantities. In addition, manufacturing challenges are likely to be compounded in the future [2,3] as more 'designer' proteins such as bispecific antibodies (bsAbs) [4] emerge as promising therapeutics [5].

The accumulation of genomic data and availability of next generation sequencing (NGS) platforms [6] has greatly accelerated the study of CHO cell biology and the information derived from these studies is beginning to have a significant impact in cell line and process development. Transcriptomics and proteomics studies have been extensively utilised (in some cases simultaneously [7]) to associate expression patterns with bioprocess performance [8]. Perhaps the most striking outcome of parallel gene and protein expression analysis is the lack of correlation between these two levels of the biological system. The function of non-coding RNAs has been shown to be responsible for a portion of the disparity observed between gene expression and protein abundance [7]. In particular, post-transcriptional regulation of gene expression via microRNAs has been widely studied in biopharmaceutical manufacturing [9]. Despite the progress made in understanding the role of miRNAs there is, however, a great deal still to learn about the fundamental principles and regulation of protein synthesis in CHO cells during the production of recombinant therapeutic proteins.

In recent years a new method, ribosome footprint profiling (RiboSeq), has rapidly expanded our understanding of translational regulation in a range of model organisms (Table 1). RiboSeq is a NGS based approach, which provides a genome-wide 'snapshot' of translation [10••]. The initial stage of RiboSeq utilises translation elongation or initiation inhibitors to arrest and fix translating ribosomes on mRNA molecules. Cells are subsequently lysed and a nuclease treatment performed. In eukaryotes, a transcript-associated ribosome shields a ~30 nucleotide footprint from digestion by the nuclease. Upon purification of the ribosomes and release of ribosome protected fragments (RPFs), a NGS library is prepared and sequenced. The quantitative data acquired from millions of actively translating ribosomes in the cell represents an indirect measurement of active translation across the genome. The technique overcomes the limitations of traditional microarray based polysome profiling by enabling the acquisition of positional information at

Table 1

Summary of selected RiboSeq experiments. For each study the species, aspect of translation being studied, inhibitor concentration and treatment time is shown. *Abbreviations*: CHX, cycloheximide; LTM, lactimidomycin; HARR, harringtonine; EME, emetine.

Study	Species	Focus	Translation inhibition		Significance
			Drug (concentration)	Time (min)	
Lee *et al.*, 2012 [17]	Human	Initiation	CHX (100 μM) LTM (50 μM) HARR (2 μg/mL)	30	Simultaneous analysis of elongation and initiation enabled the identification of alternative initiation sites
Kallehauge *et al.*, 2017 [14]	Chinese hamster	Elongation	CHX (100 μg/mL)	3	First published RiboSeq study in CHO cells.
Ingolia *et al.*, 2009 [10]	Yeast	Elongation	CHX (100 μg/mL)	2	First published RiboSeq experiment
Ingolia *et al.*, 2012 [15]	Multiple species	Elongation/ initiation	CHX (100 μg/mL) HARR (2 μg/mL)	2	Protocol for ribosome profiling of cell lines.
Ingolia *et al.*, 2011 [16]	Mouse	Elongation/ initiation	HARR (2 μg/mL) CHX (100 μg/mL) EME (20 μg/mL)	Not specified	Calculation of the average rate of translation elongation at 5.6 codons per second
Jan *et al.*, 2014 [22]	Yeast & human	ER localised protein synthesis	CHX (100 μg/mL)	2	Development of a ribosome profiling based method, which enables measurement of localised protein synthesis
Guo *et al.*, 2010 [26]	Human & mouse	Post-transcriptional regulation	CHX (100 μg/mL)	8	A study of the mechanism of action of miRNAs
Thoreen *et al.*, 2012 [45]	Mouse	Regulation of translation	CHX (conc. not specified)	5	Application of ribosome profiling to define the role of mTORC1 on translational regulation
Gerashchenko *et al.*, 2012 [24]	*Yeast*	Regulation of translation	CHX (0.1 g/L)	3	A study of the effect of hydrogen peroxide on translational regulation
Weinberg *et al.*, 2016 [46]	*Yeast*	Initiation/ elongation	Not specified	Not specified	Development of new approaches to apply ribosome footprint profiling
Yu *et al.*, 2015 [49]	*N. crassa*	Elongation	Not specified	Not specified	Application of ribosome profiling to study the effect of codon usage on the rate of translation elongation
Ingolia *et al.*, 2014 [57]	Mouse	Identification of open reading frames (ORFs)	CHX (100 μg/mL) EME (50 μg/mL)	1	Application of ribosome profiling to study translation outside annotated protein-coding regions
Chew *et al.*, 2013 [52]	Zebrafish	Identification of ORFs	Not specified	Not specified	Application of ribosome profiling to study the translational potential of long non-coding RNAs

nucleotide precision for the ribosomes associated with a particular transcript.

Mass spectrometry (MS) is a powerful platform for determining protein abundance [11•], yet despite the significant improvements in instrumentation and experimental methods achieved in recent years, only a fraction of protein products present in a cell can be detected. Although RiboSeq does not measure protein abundance directly, the technique can provide complementary information to MS based proteomics and can facilitate deep understanding of the process of translation. For instance, RiboSeq experiments have been previously utilised to refine databases for MS based proteomics [12]. Ribosome footprint profiling provides a genome wide view of active translation by identifying mRNAs associated with translating ribosomes and the ribosomal occupancy at each position within the mRNA. In addition, the technique can recognise new translation start sites, regulatory regions and detect small proteins that may not be confidently identified using mass spectrometry [13].

Following publication of the first RiboSeq study of an antibody producing CHO cell line [14••], we have now begun to interrogate a crucial layer of the biological system and study the cellular production of therapeutic proteins in exquisite detail. The information gained from RiboSeq analysis has potential to deepen our understanding of cell factories, identify new avenues for cell line engineering and uncover new rules for therapeutic protein design to maximise production rates. In this review, we present a summary of the procedures for ribosome footprint profiling. Examples of how the technique has improved our understanding of ribosomal translation are then provided along with illustrations of how the knowledge gained by RiboSeq analysis could be used to enhance biopharmaceutical manufacturing.

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