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Research review paper

Precision control of recombinant gene transcription for CHO cell synthetic biology

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ABSTRACT

The next generation of mammalian cell factories for biopharmaceutical production will be genetically engineered to possess both generic and product-specific manufacturing capabilities that may not exist naturally. Introduction of entirely new combinations of synthetic functions (e.g. novel metabolic or stress-response pathways), and retro-engineering of existing functional cell modules will drive disruptive change in cellular manufacturing performance. However, before we can apply the core concepts underpinning synthetic biology (design, build, test) to CHO cell engineering we must first develop practical and robust enabling technologies. Fundamentally, we will require the ability to precisely control the relative stoichiometry of numerous functional components we simultaneously introduce into the host cell factory. In this review we discuss how this can be achieved by design of engineered promoters that enable concerted control of recombinant gene transcription. We describe the specific mechanisms of transcriptional regulation that affect promoter function during bioproduction processes, and detail the highly-specific promoter design criteria that are required in the context of CHO cell engineering. The relative applicability of diverse promoter development strategies are discussed, including re-engineering of natural sequences, design of synthetic transcription factor-based systems, and construction of synthetic promoters. This review highlights the potential of promoter engineering to achieve precision transcriptional control for CHO cell synthetic biology.

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Contents

Abbreviations: CHO, Chinese hamster ovary; Cirp, cold-inducible RNA-binding protein; CMV-IE, cytomegalovirus immediate early; CRISPR, clustered regularly interspaced short palindromic repeats; DBD, DNA binding domain; DTE, difficult-to-express; EF1α, elongation factor 1 alpha; ER, endoplasmic reticulum; gRNA, guide RNA; hCMV-IE1, human cytomegalovirus immediate early 1; HT, high-throughput; LTR, long terminal repeat; MAb, monoclonal antibody; MAR, matrix attachment region; PIC, pre-initiation complex; SV40E, simian virus 40 early; TALE, transcription activator-like effector; TF, transcription factor; TFRE, transcription factor regulatory element; UPR, unfolded protein response; ZF, zinc finger.

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1. Introduction

The majority of new biopharmaceuticals brought to market are recombinant monoclonal antibodies (MAbs) utilizing Chinese hamster ovary (CHO) cells as a production host (Walsh, 2014). Over recent years, the creation of production CHO cell lines has ostensibly matured into a streamlined, rapid process. This is largely a consequence of the introduction of new platform technologies that permit rapid selection, isolation and testing of clonally derived subpopulations (Kuystermans and Al-Rubeai, 2015; Lai et al., 2013), and underpinning this, new genetic engineering technologies have been developed that offer general improvements in transgene expression and stability, e.g. matrix attachment regions (MARs), site-specific genomic integration, codon optimization algorithms (Kotsopoulou et al., 2010; Lee et al., 2015b; Saunders et al., 2015). Of course, recent advances in genome editing now offer new possibilities for rapid, high throughput knock-in and knock-out of functional genes (Lee et al., 2015a; Ronda et al., 2014). We also have access to CHO genomic tools and resources that are beginning to impact cellular engineering strategies (Datta et al., 2013; Hammond et al., 2012).

Despite this, bioindustrial CHO cell factory development still relies heavily upon blind screening of genetic/functional heterogeneity in parental CHO cell populations to derive a phenotypic variant capable of the core manufacturing process objective: synthesize and secrete a complex protein product whilst maintaining rapid cellular biomass accumulation. Moreover, the cell factory should maintain this functionality over many generations. Certainly we may be able to more accurately integrate the transgene in the host cell genome, and include some sequences that predispose the local genetic environment or some synthetic processes towards stable, higher-level expression, but ultimately we do not design and therefore precisely control the variable ability of the host cell environment to manufacture a specific protein. We can understand, as observed during transient gene expression, that nearly all CHO cells in a parental population can manufacture many protein architectures to some extent. However, when the cell factory is required to achieve both high-level proliferation *and* production, we require a cell factory with a set of manufacturing machinery and associated synthetic and regulatory processes particularly attuned to the idiosyncratic requirements of a given protein product.

There is one major caveat to the above where cellular product processing is *not product specific*. For years we have demonstrated control of synthetic processes involved in post-translational modification of recombinant proteins, most obviously N-glycosylation (Beck, 2013; Jedrzejewski et al., 2013). Importantly, these molecular modifications (the reason that CHO/mammalian cell factories are utilized in the first place) often govern the bioactivity and pharmacodynamics of the product in vivo (Jefferis, 2012). However, in these examples, generic CHO cell engineering yields engineered host cells that may be employed to produce many different protein products. Most often a single reaction has been eliminated (e.g. α1,6-fucosyltransferase (Yamane-Ohnuki et al., 2004)) or incorporated (e.g. β1-4-N-acetylglucosaminyltransferase III (Davies et al., 2001)), with more recent examples of co-expression of up to three processing enzymes (Yin et al., 2015). Glycosylation engineering, as an example of CHO cell engineering, has been very successful. However, this is the low hanging fruit. We generally know what to engineer in a digital on/off sense, and we do not have to deal with the biological variability of protein product architecture and regulation of transfected cell clone synthetic processes — an interface that largely defines product manufacturability.

We argue that the future of CHO cell engineering has to be based on an ability to interactively design (i.e. with respect to a specific product and specific CHO host cell genotype) and create new CHO cell functional phenotypes that do not exist naturally. We will need to extend the CHO cell "design space" beyond the natural boundaries created by random genetic mutation and chromosomal instability. Historically, through iterative improvement we have succeeded in improving the design of an external, multi-component synthetic environments for CHO cells (e.g. media/feed/process composition), which have massively improved functional performance (Wurm, 2004; Zhu, 2012). To control complex multigenic phenotypes (e.g. increased cell growth rate) and product-specific manufacturing capability, we now need to create a technology platform that enables an internal, multi-component synthetic cell environment for knowledge-based control of cell factory manufacturing performance.

This will require a CHO cell engineering platform that has one core practical attribute: the coordinated expression of multiple transgenes at precise relative stoichiometry. For instance, how do we co-express eight functional proteins in a host cell at a relative stoichiometry optimal for a new metabolic pathway function that we wish to introduce? This is a fundamental operational requirement of any biological or indeed engineered system — try making a cake using a random proportion of known ingredients! Although recent reports describe new methods for synthetic multigene vector construction for mammalian cell synthetic biology (Guye et al., 2013; Kriz et al., 2010), the synthetic parts or positional combinations that may be utilized on the vector to achieve a given stoichiometry of encoded proteins are typically not dealt with. We will need to create bespoke, synthetic mammalian cell vectors that not only harbour multiple genetic components, but also enable them to operate at an optimal, designed stoichiometry.

In a bioproduction context, embedding the relative stoichiometry of multiple transgenes is entirely desirable. The simplest example would be expression of heavy and light chain genes at an optimal ratio for synthesis of a given MAb, where the optimal ratio may be very MAb specific (Ho et al., 2013; Pybus et al., 2014; Schlatter et al., 2005). For more advanced applications, to achieve a significant change in cell factory production capability may require unnatural combinations of functional genes (Le Fourn et al., 2014; McLeod et al., 2011; O'Callaghan et al., 2010; Xiao et al., 2014) that may be designed to introduce a single concerted function (e.g. a new metabolic pathway) or used to simultaneously engineer different functional modules of a cell. Indeed, as industrial pipelines fill with engineered protein products it is entirely likely that protein-specific solutions may be necessary. For example, we recently showed for both IgG1 MAbs exhibiting variation in production titre and an engineered difficult-to-express fusion protein that different combinations of functional proteins known to modulate cellular folding/assembly capacity could, in a protein-specific manner, increase production titre significantly (Johari et al., 2015; Pybus et al., 2014). Moreover, the background host cell or production process context may significantly alter required functional genes and their relative stoichiometry. To enable significant reductions in development times for these new products, there is an urgent need to shift from screening-led to design-led technologies; embedding prediction and design of product manufacturability at an earlier stage in bioprocess development to speed products into the clinic.

1.1. Optimized stoichiometry of genetic components in synthetic CHO cell factories can be achieved via promoter engineering

CHO cell engineering still relies upon the widespread use of a very limited set of complex, functionally ill-defined genetic components. Currently available technologies generally employed in industry only enable gross control of recombinant gene expression. We require new design and engineering technologies that will enable us to equip cells with new machinery and processing capability optimally suited for a specific intended purpose. In essence this is a statement of the synthetic biology paradigm (Church et al., 2014; Silver et al., 2014), applied as context-dependent retro-fitting. This process will be dominated by two key questions, which new functionalities are required (for a given product/cell line/process) and how do we embed optimal performance?

Transcriptional-control tools are an attractive route to achieving the essential optimal stoichiometry of biological parts as i) transcription is

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