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Recombinant protein production from stable mammalian cell lines and pools

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We highlight recent developments for the production of recombinant proteins from suspension-adapted mammalian cell lines. We discuss the generation of stable cell lines using transposons and lentivirus vectors (non-targeted transgene integration) and site-specific recombinases (targeted transgene integration). Each of these methods results in the generation of cell lines with protein yields that are generally superior to those achievable through classical plasmid transfection that depends on the integration of the transfected DNA by non-homologous DNA end-joining. This is the main reason why these techniques can also be used for the generation of stable cell pools, heterogenous populations of recombinant cells generated by gene delivery and genetic selection without resorting to single cell cloning. This allows the time line from gene transfer to protein production to be reduced.

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Introduction

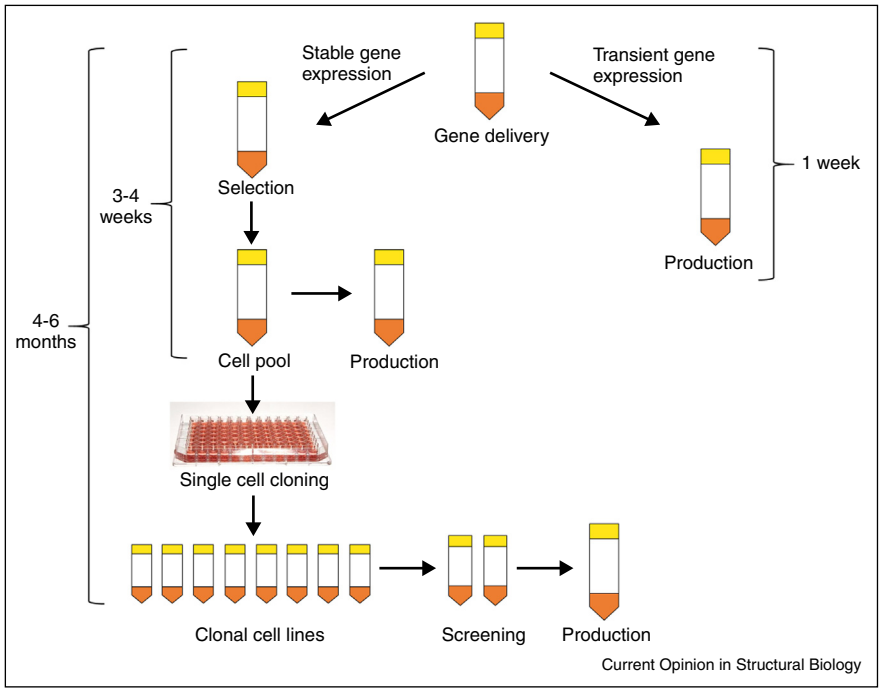
When considering mammalian cells for the production of recombinant proteins for structural studies, the predominant choice is the transient transfection of human embryonic kidney (HEK293) cells [1,2]. Transient gene expression in suspension-adapted HEK293 cells is rapid and often generates adequate amounts of protein (Figure 1). Transient gene expression usually requires more than 1 mg of plasmid DNA per liter of transfection [2]. For projects which require large culture volumes, plasmid DNA preparation may become a limitation, although efforts are underway to reduce the plasmid DNA amount while maintaining high protein yields [3]. Nevertheless, this point needs to be taken into consideration when planning a project. As an alternative to transient protein production, stable gene expression from a recombinant cell line has several advantages including a substantially reduced need for plasmid DNA, a greater ease of volumetric scale-up, and a renewable source of the recombinant cell line from a frozen cell bank. The main drawback of stable gene expression is the time required from gene delivery to protein production (Figure 1). With the gene delivery methods described here, it is now worth considering protein production from a stable cell pool rather than a clonal cell line (Figure 1) [4••]. This approach eliminates the need for single cell cloning and cell line screening, the most time-consuming steps in cell line development. Besides summarizing recent advances in cell line and cell pool generation, we provide brief overviews of the cultivation of mammalian cells in suspension and of the design of vectors for stable gene expression. For additional information on proteins whose structures have been resolved following production in mammalian cells by stable or transient gene expression, there are several recent reviews on the subject [1,2,5••].

Host cells and their cultivation in suspension

The most popular hosts for stable gene expression are suspension-adapted HEK293 and Chinese hamster ovary (CHO) cells. There are several reasons for their application to structural studies: (i) they grow to high cell densities in suspension, (ii) they are easily transfected, (iii) a broad range of serum-free media are commercially available for their cultivation, (iv) there are glycosylation-defective strains (HEK293S-GnTI$^-$, HEK293GlycoDelete, and multiple CHO lec1 strains) [6–8], (v) there are strains that stably overexpress the tetracycline repressor (TetR) for induced expression (T-REx®-293 and T-REx™-CHO), and (vi) there are strains for the targeted integration of transgenes (Flp-In™-293, Flp-In™ T-REx®-293, and Flp-In™-CHO). Other cell lines such as HeLa, baby hamster kidney (BHK), and human PER.C6 can also be grown in suspension, and the methods for stable cell generation described below are also relevant to these hosts.

Based on our own experience, CHO is superior to HEK293 for the generation of stable cell lines for the production of transmembrane and secreted proteins. Moreover, since CHO cells are the main host for the large-scale production of therapeutic proteins, considerable effort has gone into enhancing their productivity

Figure 1



Summary of stable and transient gene expression strategies with suspension-adapted mammalian cells. Transient gene expression involves the transfection of cells with one or more expression vectors. The cells are then maintained in culture until the end of the production phase (1–10 days). Stable gene expression involves the transfection of cells with one or more expression vectors followed by a selection phase of 1–2 weeks. The recombinant cells which survive selection can be maintained as a cell pool for protein production or can be used for single-cell cloning to generate stable cell lines. A number of clonal cell lines are eventually screened to obtain one or more with superior protein productivity, cell growth in suspension, and stability of protein production over time in the absence of selective pressure. One of the clonal cell lines is then used for recombinant protein production. The time lines for the generation of stable cell lines and cell pools are approximately the same regardless if the recombinant gene(s) are integrated into the host genome using a transposon, lentivirus vector, or recombinase. Use of the targeted and non-targeted integration methods described here are expected to increase the efficiency of cell line generation by reducing the number of clonal cell lines that need to be screened to find a one that is satisfactory. In addition, application of these gene delivery methods to cell pool generation provides a rapid and cost-effective approach to protein production.

through host engineering [9]. Improving cell growth and viability by overexpression of either mTOR or the anti-apoptosis protein Bcl-X_L resulted in higher yields of recombinant antibodies and cell surface receptors, respectively [10,11]. Targeting of the protein secretory pathway improved secreted protein yields [12], and the overexpression of the transcription factor YY1 resulted in higher recombinant antibody yields in multiple HEK293 and CHO strains [13•]. Alternatively, either the overexpression or depletion of specific CHO microRNAs (miRNAs) proved to be effective approaches to enhancing secreted protein yields [14•,15]. To support host cell engineering, a vector for inducible transgene expression in addition to the constitutive overexpression of multiple short-hairpin RNAs for the knockdown of several cellular target genes by RNA interference was recently described [16].

For the suspension cultivation of HEK293 and CHO cells, most laboratories are using orbitally shaken containers such as Erlenmeyer flasks, cylindrical and squared glass bottles, and Nalgene carboys [17]. In addition, TubeSpin® bioreactor 50 and 600 tubes, 50-mL and 600-mL centrifugation tubes with a vented cap, have been developed for working volumes of 2–20 mL and 100–500 mL, respectively [17,18]. For cultures of 10–200 L, disposable orbitally shaken bioreactors of 50-L and 200-L have recently been introduced [19,20]. Disposable WAVE bioreactor bags for culture volumes starting from 100 mL remain a popular alternative choice [21].

Expression vector elements

For stable cell lines, transgene silencing is one of the most important concerns since protein production campaigns may require several weeks of cell cultivation in the absence of selective pressure. Although most mammalian expression vectors rely on transgene transcription from the human cytomegalovirus major immediate early promoter/enhancer (hCMV-MIE), this is not the best choice for stable gene expression since it is vulnerable to gene silencing in many cell lines including CHO [22,23••]. The risk of silencing may be reduced by incorporating a matrix associated region (MAR), ubiquitously-acting chromatin