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Current Opinion in Biotechnology

The genome editing toolbox: a spectrum of approaches for targeted modification

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The increase in quality, quantity, and complexity of recombinant products heavily drives the need to predictably engineer model and complex (mammalian) cell systems. However, until recently, limited tools offered the ability to precisely manipulate their genomes, thus impeding the full potential of rational cell line development processes. Targeted genome editing can combine the advances in synthetic and systems biology with current cellular hosts to further push productivity and expand the product repertoire. This review highlights recent advances in targeted genome editing techniques, discussing some of their capabilities and limitations and their potential to aid advances in pharmaceutical biotechnology.

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Current Opinion in Biotechnology 2014, **30**:87–94

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.2014.06.005

Introduction

Achieving mg/L quantities of an antibody can easily be achieved by introducing a transgene into a host cell. However, the production of gram quantities (upwards to tens of grams per liter) requires a combination of cell line engineering, expression optimization, and advanced culturing control. As we continue to push the envelope for titer, we require increasingly precise methods to modify the genome. Yet, until recently, the genetic tools available to make targeted edits exhibited rather coarse resolution even for model organisms.

Enabling targeted editing of eukaryotic and mammalian genomes diversifies the biopharmaceutical repertoire and offers design of selection systems beyond current schemes (e.g. dihydrofolate reductase-deficient CHO cell lines or antibiotic resistance). Metabolic engineering of mammalian cells to produce the first bioengineered heparin is imminent [1]. Furthermore, combining pathway understanding with well-characterized synthetic genetic parts will enable additional complex products to be produced. These efforts will be greatly expedited by targeted genomic edits. Moreover, these technologies open up the possibility to engineer host cell lines without selection markers (or in some cases, for marker-less genome editing), thus bypassing antibiotic selection schemes that can be suboptimal depending on the selecting agent used [2•].

With the increasing synthetic toolkit for genetic editing, cell line development is about to experience a renewal at the intersection of systems biology and synthetic biology. In this review, we discuss recent advancements in a spectrum of targeted genome editing tools ranging from coarse, loci-level resolution to precise, base-pair specific modifications. In particular, we discuss the capabilities and drawbacks of transposons, group II introns, recombinases, and nucleases, especially in the context of cell lines relevant for biopharmaceutical production. We conclude with a brief assessment of the potential for these genome editing tools to aid pharmaceutical biotechnology and expedite therapeutic candidate development.

Expansion and evaluation of targeted genome editing techniques

High-throughput analytical methods and cheap genome sequencing enable a more precise linkage between genotype and phenotype. These approaches could theoretically allow researchers to make concerted and rational decisions about the genotype of a cell line. This capacity to move beyond random libraries and integrations (or at the very least, understand why and how a cell line performs as it does in an effort to recapture high productivity for another molecule), require a sophisticated suite of genome editing tools. Moreover, the complexity across different cell types and cell lines makes such a vision challenging. A variety of techniques (Figure 1) have been developed to edit genomes including adapting components from other species (e.g. transposases, recombinases, group II introns, and RNA-directed nucleases) and creating synthetic approaches comprised of discrete building blocks (e.g. zinc-finger and transcription activator-like effector nucleases). This spectrum of targeted genome editing tools has a myriad of capabilities and applications, but each approach has distinct drawbacks

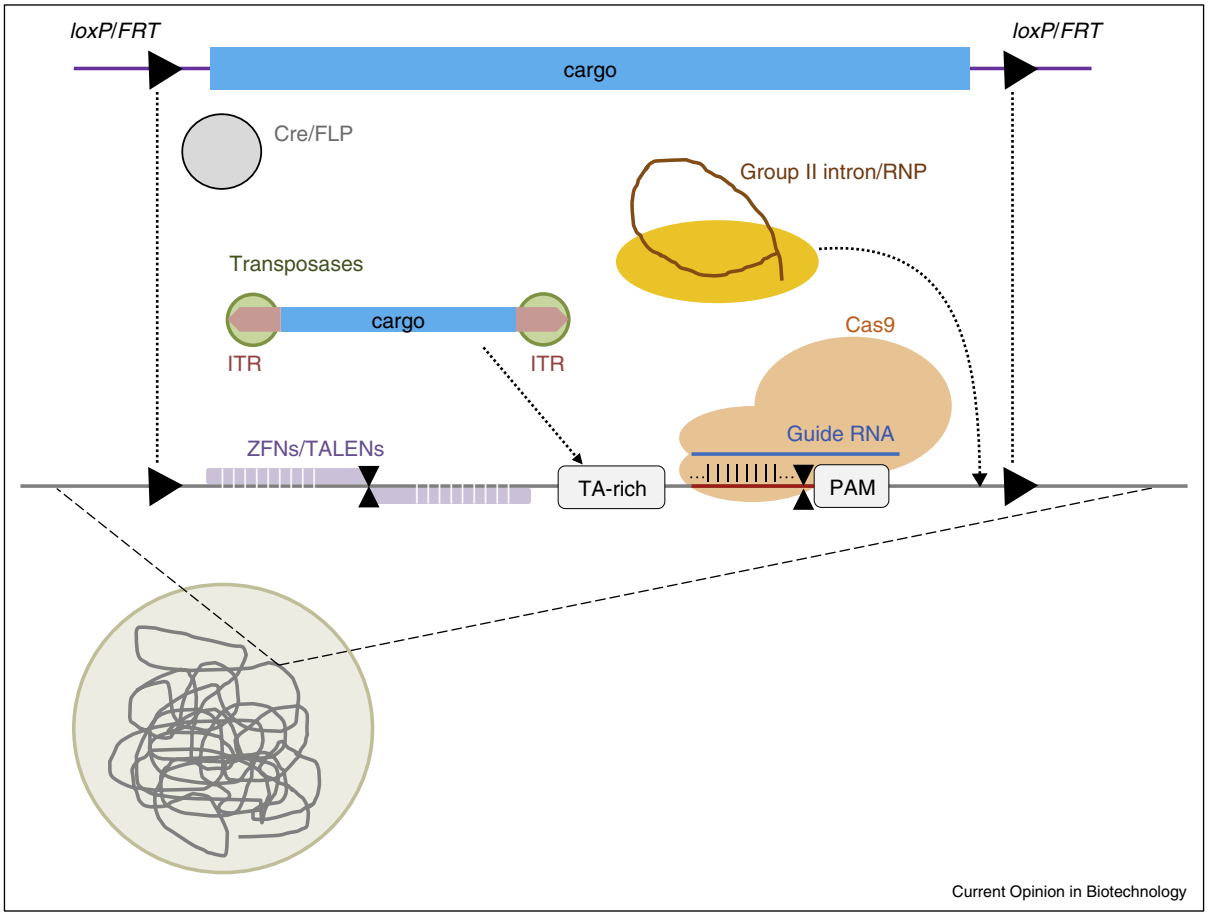
and limitations (Table 1). In the sections that follow, we briefly discuss the use and development of these techniques in recent years and how they can impact pharmaceutical biotechnology.

Large genetic elements

Transposable elements, such as transposons and group II introns, and recombinases were among the first genome editing tools applied to mammalian cell lines. Transposons, group II introns, and recombinase-mediated cassette exchanges link desired genetic cargo as part of the mobile element with recognition sites flanking this cargo (Figure 1). Transposon-aided insertion is mediated by sequence recognition rules (e.g. TA-rich region) that vary depending on the transposable element. Self-splicing group II introns target DNA with the same or similar sequences to the donor sequence containing the intron and incorporate the intron sequence through reverse transcription. Lastly, recombinases recognize specific DNA sequences (e.g. *loxP* and *FRT*) between which exchanges or rearrangements occur.

In the context of pharmaceutical applications, transposable elements (for an active list in vertebrates, see review by Ivics [3]) such as *Sleeping Beauty* (*SB*) and *PiggyBAC* (*PB*) can serve as a safe alternative to viral vectors for gene delivery (Table 1) [4]. Such systems require expression of the transposase and can deliver genetic cargo to pre-set loci due to particular sequence preferences and genomic integration patterns of the transposases [3]. As an example, functional wild-type heme oxygenase-1 was delivered to the livers of mice inflicted with sickle cell disease using a *SB* transposase plasmid [5] and this *SB* system recently showed a positive outlook for a human

Figure 1



Targeted genome editing spans coarse, regional locus-level recognition to nucleotide-level specificity. Targeted genome editing tools permit manipulation of a variety of host cells relevant to the biopharmaceutical industry. The combination of the tools described in this review improves prospects for engineering in these cells. Transposases recognize ITR sequences flanking the DNA cargo and mediate movement of the cargo elsewhere in the genome (e.g. TA-rich regions). Recognition sites for recombinases such as *loxP*/*FRT* are specified as DNA sequences. Group II introns require additional proteins (e.g. reverse transcriptase) to fully carry out their targeted integration into the genome. The target DNA sequence is sufficient for inducing specific cleavage by nucleases; however, the guide RNA (gRNA) is required to direct Cas9 nuclease to the target site including the PAM. Abbreviations and symbols: ɪ denote the targeted cleavage site triggered by the nuclease. ZFN, zinc-finger nuclease. TALEN, transcription activator-like effector nuclease. PAM, protospacer adjacent motif. ITR, inverted terminal repeats. RNP, ribonucleoprotein.