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Review

Recombinase-mediated cassette exchange (RMCE) — A rapidly-expanding toolbox for targeted genomic modifications

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ABSTRACT

Starting in 1991, the advance of Tyr-recombinases Flp and Cre enabled superior strategies for the predictable insertion of transgenes into compatible target sites of mammalian cells. Early approaches suffered from the reversibility of integration routes and the fact that co-introduction of prokaryotic vector parts triggered uncontrolled heterochromatization. Shortcomings of this kind were overcome when Flp-Recombinase Mediated Cassette Exchange entered the field in 1994. RMCE enables enhanced tag-and-exchange strategies by precisely replacing a genomic target cassette by a compatible donor construct. After "gene swapping" the donor cassette is safely locked in, but can nevertheless be re-mobilized in case other compatible donor cassettes are provided ("serial RMCE"). These features considerably expand the options for systematic, stepwise genome modifications. The first decade was dominated by the systematic generation of cell lines for biotechnological purposes. Based on the reproducible expression capacity of the resulting strains, a comprehensive toolbox emerged to serve a multitude of purposes, which constitute the first part of this review. The concept *per se* did not, however, provide access to high-producer strains able to outcompete industrial multiple-copy cell lines. This fact gave rise to systematic improvements, among these certain accumulative site-specific integration pathways. The exceptional value of RMCE emerged after its entry into the stem cell field, where it started to contribute to the generation of induced pluripotent stem (iPS-) cells and their subsequent differentiation yielding a variety of cell types for diagnostic and therapeutic purposes. This topic firmly relies on the strategies developed in the first decade and can be seen as the major ambition of the present article. In this context an unanticipated, potent property of serial Flp-RMCE setups concerns the potential to re-open *loci* that have served to establish the iPS status before the site underwent the obligatory silencing process. Other relevant options relate to the introduction of composite Flp-recognition target sites ("heterospecific *FRT*-doublets"), into the LTRs of lentiviral vectors. These "twin sites" enhance the safety of iPS re-programming and -differentiation as they enable the subsequent quantitative excision of a transgene, leaving behind a single "*FRT*-twin". Such a strategy combines the established expression potential of the common retro- and lentiviral systems with options to terminate the process at will. The remaining genomic tag serves to identify and characterize the insertion site with the goal to identify genomic "safe harbors" (GOIs) for re-use. This is enabled by the capacity of "*FRT*-twins" to accommodate any incoming RMCE-donor cassette with a compatible design.

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Abbreviations: attB/*att*P, recombinase-attachment sites within the bacterial and phage genomes; ccc, covalently-closed circular; Cre, cyclization recombinase/causes recombination; DSB, double-strand break; ESC, embryonic stem cell; flexing, Flp-mediated excision; EUCOMM, European Conditional Mouse Mutagenesis program; flirted, *FRT*-flanked; floxed, flanked by *lox*Ps; Flpe, enhanced (temperature-adapted) version of Flp; Flp, flippase; Flpo, codon-optimized version of Flpe; *FRT*, Flp-recombinase target; GEMMs, genetically engineered mouse models; HR, homologous recombination; iCre, improved (codon-optimized) version of Cre recombinase; IR, illegitimate recombination; INT, integrase; INV, invertase; *lox*P, locus of crossover in phage P1; MOI, multiplicity of (viral) infection; NHEJ, non-homologous end joining; *puro*, transcription unit encoding the Puromycin selection marker; RDF, recombination directionality factors; RES, resolvase; RMCE, recombinase-mediated cassette exchange; RMDI, Recombinase-Mediated DNA Insertion; RMGR, recombinase-mediated genome replacement; RPT, retrovirus-mediated protein transfer; RT/RTS, recombinase target/-target-site; SIDR, SerINT-mediated directional recombination; S/MAR, scaffold/matrix attachment element; SSR, site-specific recombinases/-recombination; UCOE, universal chromatin opening element (from a bidirectional promoter).

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1. Introduction

Exemplified by integrating viral vectors, the insertion of therapeutic transgenes in the human genome has been identified as one trigger of unanticipated consequences like clonal cell amplification and leukemia. The growing awareness that, depending on the number and assembly of inserts, the ultimate structure of the integration *locus* may influence the level and consistency of transgene expression led to a variety of novel concepts to account for these complications.

A strategy to control position effects and transgene cross-interactions is to provide transcription units with genomic bordering elements, among these scaffold/matrix attachment regions (S/MARs) and insulator elements. A somewhat related solution relies on the use of extended BAC-based vectors. Being autonomous expression units these prove to free from position effects. These approaches, however, could not overcome concerns that multiple transgene copies per cell might have adverse phenotypic consequences. More advanced strategies rely on improved transduction routes such that the recipient cells do not suffer from transgenes residing at particular or several different integration sites.

Initiated two decades ago a pilot approach emerged from random gene trapping by retroviral insertion (Friedrich and Soriano, 1991). The trap vectors comprised a reporter gene, which remains unexpressed unless it is integrated into the first (sometimes also the second) intron of a given ES-cell gene. The construct, a self-inactivating (SIN-) vector with a splice acceptor (SA)-β-galactosidase unit in reverse orientation (ROSA-β-Gal) became the founding member of an ever growing family of "ROSA"-derivatives. One of the early mouse gene trap lines, "ROSA26", enabled ubiquitous expression of the reporter gene during embryonic development and gained relevance as a marker line in chimera experiments. Once its β-gal expression potential was firmly established for various tissues and hematopoietic cells, the ROSA26 *locus* became the first reference site with apparent genomic "safe harbor" properties. Nowadays its characteristics appear to contradict the stringent present safe harbor criteria (Section 3.2.9) as three transcripts go back to a single promoter, two of these devoid of a clear-cut ORF. The third transcript arises from the reverse strand and provides antisense properties relative to one of the non-coding transcripts. This complex arrangement of functions may explain that the *locus* evades the host's genome defense and silencing systems.

To be in the position to control the fate of transgenes with regard to copy numbers, orientation and rearrangements, early approaches relied on homologous gene targeting with host cell-dependent, but mostly minor efficiencies. Later on these efforts were supported by engineered proteins able to recognize target DNA sequences and to initiate double stranded breaks to trigger cellular DNA repair responses. Among these are Zinc-finger nucleases (ZFNs; Townsend et al., 2009), meganucleases (Pâques and Duchateau, 2007) and TAL effector nucleases (TALENs; Christian et al., 2010). Today any of these techniques or their combination permits the analysis of diverse aspects of gene function *in vivo* as it has become possible to engineer specific alterations ranging from point mutations to extended genomic deletions as well as directed chromosomal translocations.

1.1. HR versus SSR

Since the development of embryonic stem cell (ESC-) technologies in the early 1980s, which led to the generation of the first knockout mice