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Selfish DNA: a pharmaceutical perspective

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Dedicated to Prof. Dr. Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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Almost 25 years ago, Theo Dingermann published the discovery of a new mobile genetic element in the unicellular microbe *Dictyostelium discoideum* in the journal *Science*. An interesting property of this new molecular parasite, the *Dictyostelium* Repetitive Element (DRE), was that all integrations were found approximately 50 base pairs (bp) upstream of transfer RNA (tRNA) genes in the *D. discoideum* genome, thus implying an active targeting mechanism to avoid the disruption of host cell genes by the retrotransposition process. Since then, the facultative multicellular "social amoeba" *D. discoideum* has become a popular model for analyzing complex cellular functions such as cell movement, chemotaxis, phagocytosis, and cell differentiation, important areas of biomedical research that are often hard to investigate in cells from "higher organisms" including humans. Therefore, progress in the development of methods to study *Dictyostelium* biology has also provoked research on transposable elements in this organism. Early work on the DRE element suggested that studying its molecular mechanism of site-specific integration might promote human gene therapy technology through the design of integrating gene transfer vectors with low intrinsic genotoxic potential. In this review article, I will briefly review the original research performed on the DRE transposable element in the Dingermann lab and report on how the emergence of genomics technologies and the development of tools to analyze *de novo* retrotransposition events in *D. discoideum* cells will expand our knowledge of DRE biology in the future.

1. Introduction

In the 1940 s, Barbara McClintock studied a prominent chromosome breakage locus in maize. She observed that the agent causing the chromosome breaks was able to change position within maize chromosomes, leading to unstable mutations (McClintock 1948). McClintock named this phenomenon "transposition", and today we recognize the chromosome-breaking agents as transposons or mobile DNA elements. Although the significance of McClintock's discovery was largely ignored by the scientific community for many years, mobile elements were then discovered in other organisms and today we know that virtually all genomes accommodate mobile elements to a certain extent. Since this discovery, a discussion has continued regarding the possible impact of transposable elements on host cell fitness and evolution. It has been argued that transposable elements are molecular parasites that invade genomes and try to increase in copy number over time without serving any beneficial functions to the host (Doolittle and Sapienza 1980; Orgel and Crick 1980). Thus, transposable elements can be considered as pieces of selfish DNA that may be harmless at best but potentially disastrous if transposition leads to genome instability resulting from insertional mutagenesis, non-allelic homologous recombination, or induction of chromosome breaks (Hedges and Deininger 2007). The possible consequences are best exemplified by the observation of nearly 100 disease-causing insertions of transposable elements in the human genome (Hancks and Kazazian 2012). Despite the destructive potential that mobile elements certainly have, there is convincing evidence that mobile elements have positively contributed to genome evolution as a consequence of host-transposon coevolution. For example, transposable elements may alter the expression of genes near the integration site or shuffle genomic DNA from one site to another during transposition, eventually creating entirely new genes. Host cells have even adapted protein functions from their invaders to serve essential cellular functions, such as the RAG-1/RAG-2 system for the recombination of antibody genes or the telomerase enzyme that maintains intact chromosome ends in most organisms (Cordaux and Batzer 2009; Goodier and Kazazian 2008; Levin and Moran 2011).

There are two major groups of transposable elements. The DNA transposons, or class II elements, move by a "cut-and-paste" mechanism, meaning that they remove themselves from certain chromosomal loci to insert elsewhere. Barbara McClintock's chromosome-breaking agent belongs to this class of elements. Class I elements are also referred to as retrotransposons. They do not typically move throughout the genome but reverse-transcribe their genomic RNA into DNA and subsequently integrate the copies at new genomic positions ("copy-and-paste" mechanism).

Here, I will briefly review the discovery of the TRE family of retrotransposons in the "social amoeba" *Dictyostelium discoideum*. TRE retrotransposons have a strict association with tRNA genes, which is mediated by active recognition of tRNA genes and prevents gene disruptions in the highly gene-dense *D. discoideum* genome (Winckler et al. 2011). From a pharmaceutical perspective on the development of human gene therapy,

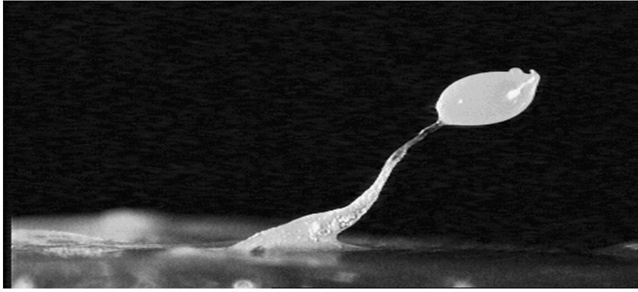

Fig. 1: A fruiting body of *Dictyostelium discoideum*

evaluating the molecular mechanism of the highly specific integration site selection by *Dictyostelium* transposable elements may lead to new technology that reduces the genotoxicity of integrating gene transfer vectors.

2. *Dictyostelium* – renaissance of a model organism

D. discoideum is a unicellular, amoeboid organism that lives in the soil and feeds on other microorganisms such as bacteria and yeasts. An interesting property of *D. discoideum* is that under environmental conditions that are unfavorable for growth, single amoebae can aggregate to form multicellular structures that eventually form fruiting bodies (Fig. 1). Multicellular development of *D. discoideum* has been well studied biochemically and genetically, and extensive intercellular communication, chemotactic cell movement, changes in global gene expression in response to external stimuli, and cell differentiation have all been found. These phenomena also occur in human cells, making *D. discoideum* a valuable model organism. Of course, one could ask whether it makes sense to use a "simple" microbe such as *D. discoideum* as a model in biomedical research given the long evolutionary distance between amoebae and humans. However, *D. discoideum* is in some aspects more related to metazoans than to other protists, and looking back into the history of molecular biology and genetics, it is evident that choosing a suitable "simpler" model organism is key to understanding (patho)physiological processes.

To highlight the potential of *D. discoideum* as a model organism, decades of research on cell migration and chemotaxis in *Dictyostelium* revealed close analogy to human leukocytes, which established *Dictyostelium* as a model to better understand cell motility-related pathologies of the immune and neurological systems (Carnell and Insall 2011). Dingermann and coworkers elucidated the teratogenic potential of valproic acid using the multicellular development of *D. discoideum* as a model system (Tillner et al. 1998). This inspired an in-depth analysis of complex changes in intracellular signaling evoked by valproic acid treatment in developing *D. discoideum* cells. The results of these studies have led to a better understanding of bipolar disorder and may eventually contribute to the development of new therapeutic treatments (Ludtmann et al. 2011). Sequencing of the *D. discoideum* genome (Eichinger et al. 2005) has revealed the presence of a large number of genes that have high similarity with human genes that may be involved in pathological processes. Thus, *D. discoideum* appears to be a simple, genetically tractable model to study key aspects of the molecular pathophysiology underlying prominent disorders such as Alzheimer's disease (McMains et al. 2010), Parkinson's disease (Gilsbach et al. 2012), Huntington's disease (Myre et al. 2011; Wang et al. 2011), and several others. Further, *D. discoideum* cells are professional phagocytes that resemble human macrophages. As such, *D. discoideum* is also an outstanding model to study host interactions with important intracellular human pathogens such as *Legionella* (Balest et al. 2011; Farbrother et al. 2006; Hägele et al. 2000; Otto et al. 2004), *Mycobacterium* (Hagedorn et al. 2009; Hagedorn and Soldati 2007; Solomon et al. 2003), *Pseudomonas* (Cosson et al. 2002; Pukatzki et al. 2002), *Salmonella* (Jia et al. 2009), *Yersinia* (Vlahou et al. 2009), and others (see also a recent review by Steinert 2011).

3. The *Dictyostelium* repetitive element (DRE)

3.1. A short history of DRE discovery

In the 1980 s, Theo Dingermann studied the regulation of tRNA genes in the model organism *D. discoideum* at the University of Erlangen (Dingermann et al. 1987, 1988, 1989a). This research period was expedited by the emergence of molecular biology studies in *Dictyostelium*, and Dingermann made important contributions to the field, such as the use of β-galactosidase expressed from developmentally regulated promoters for *in situ* staining of different cell types in multicellular stages of *Dictyostelium* development (Dingermann et al. 1989b).

Dingermann's group isolated tRNA genes from the *D. discoideum* genome and sequenced their flanking regions, motivated by the idea that flanking sequences of tRNA genes might be involved in their transcriptional regulation. The group found snippets of identical DNA sequences both upstream and downstream of tRNA genes that were regarded as tRNA gene-associated repetitive elements. They further noted the presence or absence of these sequences at homologous tRNA gene loci in different strains of *D. discoideum*, suggesting that the repetitive DNA elements were in fact mobile genetic elements. This important observation was published in *Science* in 1989 (Marschalek et al. 1989) and was the starting point for a series of elegant experiments showing that the *Dictyostelium* repetitive element (DRE), as it was named, integrates ~50 bp upstream of tRNA genes and that approximately one half of all tRNA genes in the *D. discoideum* genome may be occupied by flanking DRE sequences (Hofmann et al. 1991; Marschalek et al. 1992a, b). It was immediately clear that DRE is a retrotransposon whose target site preference resembles that of certain yeast retrotransposons. However, as Dingermann noted, "The reason for this tight association is unclear" (Marschalek et al. 1989).

3.2. The Dictyostelium genome is an inhospitable environment for mobile elements

As mentioned earlier, *D. discoideum* is an important model for biological and biomedical research. Consequently, the genome of *D. discoideum* has been sequenced and was presented in *Nature* in 2005 (Eichinger et al. 2005). Characteristic features of the *D. discoideum* genome are its high gene density and low GC content of 22% (Eichinger et al. 2005). In fact, approximately 65% of the 34 Mb genome contains protein-coding genes, and intergenic regions are often less than 1,000 bp in length. Considering that the short intergenic regions must accommodate functional gene regulatory sequences such as promoters, it is evident that transposable elements invading such a compact genome must avoid disrupting genes in order to establish an active population of elements (Winckler et al. 2011).

Genome sequencing has revealed that the DRE element originally isolated by Dingermann and coworkers is one member of a family of retrotransposons that integrate either 5' (upstream) or 3' (downstream) of tRNA genes at well-defined distances (Glöckner et al. 2001). Following the discovery of the new elements, a new nomenclature was required, leading to the renaming of the DRE element as "TRE5-A" (for tRNA gene-associated retroelement) (Szafranski et al. 1999). The designation "TRE5" is used for elements that integrate ~50 bp

upstream of tRNA genes, whereas “TRE3” is used for elements that integrate ∼100 bp downstream of tRNA genes. It is worth noting that the “Tdd-3” element first described by Dingermann et al. (Marschalek et al. 1989) belongs to the TRE family and is now referred to as TRE3-A (Szafranski et al. 1999).

TRE elements contribute the vast majority of mobile element loci in euchromatic regions of the *D. discoideum* genome, demonstrating that these elements have been exceptionally successful in capturing space between protein-coding regions. They may do so without causing insertional mutagenesis by targeting flanking regions of one of the approximately 390 tRNA genes (Eichinger et al. 2005) in the *D. discoideum* genome. However, this conclusion may be biased by the examination of “old” integrations in the modern *D. discoideum* genome because *D. discoideum* cells are haploid, and cells that experience harmful insertions may be lost. In other words, it is not clear whether the integration preference of TRE retrotransposons is in fact as strict as that suggested by current genome analysis (for a more detailed discussion, see a recent review by Winckler et al. 2011).

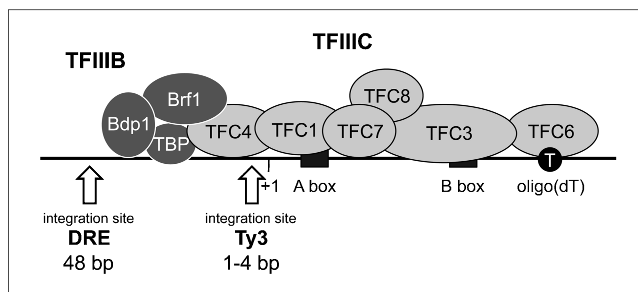

Fig. 2: Topology of the TFIIIC/TFIIIB transcription complex. Schematic of the TFIIIB/TFIIIC transcription factor complex on a canonical tRNA gene, based on a model proposed by Huang and Maraia (Huang and Maraia 2001). The intragenic tRNA promoter elements (A box and B box) and the oligo(dT) transcription termination motif of the tRNA gene are shown. The first nucleotide of the mature tRNA is indicated as + 1. The integration sites of TRE5-A and yeast Ty3 approximately 48 bp and 2–3 bp, respectively, upstream of tRNA genes are indicated

3.3. A hypothesis of tRNA gene recognition by TRE5-A elements

An in-depth analysis of the complete *D. discoideum* genome sequence revealed that 102 TRE5-A integrants are located 37–211 bp upstream of tRNA genes scattered on all six chromosomes. Sixty one percent of TRE5-A integrations were found 48 ± 3 bp upstream of tRNA genes. This is in good agreement with original work on DRE elements, in which tRNA gene loci were cloned out of the *D. discoideum* genome, and the associated DRE elements were found 50 ± 3 bp upstream of tRNA genes (Hofmann et al. 1991; Marschalek et al. 1989). Analysis of *de novo* integrants of TRE5-A into the “TRE trap” (see paragraph 3.4.) also revealed a distance of 48 ± 3 bp (n = 31) to the target tRNA gene (Beck et al. 2011; Siol et al. 2006), suggesting that this value represents the natural integration preference of TRE5-A, whereas somewhat longer or shorter distances determined for “old” integrants seem to reflect co-integration or post-integration genome rearrangements (Siol et al. 2006).

TRE5-A belongs to the subgroup of non-long terminal repeat (non-LTR) retrotransposons and is fundamentally different both in structure and retrotransposition mechanism from the Ty LTR retrotransposons that also target tRNA genes in yeasts (Chalker and Sandmeyer 1992). Thus, it seems clear that TRE5-A and the yeast Ty elements independently evolved molecular mechanisms to recognize tRNA genes as integration sites. However, there are obvious parallels, such as the absence of conserved DNA sequences in the flanking regions of tRNA genes that would suggest DNA sequence-dependent integration. A hypothesis to explain the tRNA gene specificity of TRE5-A and yeast Ty retrotransposons is that the mobile elements facilitate protein contacts with unique transcription factors that mediate the transcription of tRNA genes by RNA polymerase III (pol III). The multi-subunit transcription factor TFIIIC (Fig. 2) binds first to the intragenic promoter elements of tRNA genes, the A box and B box, in a sequence-specific manner. DNA-bound TFIIIC then mediates the binding of the transcription initiation factor, TFIIIB (Schramm and Hernandez 2002). Susan Sandmeyer’s group showed that the presence of TFIIIB subunits TBP and Brf1 (Fig. 2) is sufficient to mediate specific integration of yeast Ty3 upstream of tRNA genes, while the third TFIIIB subunit Bdp1 is not essential but enhances Ty3 integration (Yieh et al. 2002, 2000). Thus, it is possible that TRE5-A elements recognize the pol III transcription factors TFIIIC and/or TFIIIB similar to yeast Ty3. Considering the presumed topology of the TFIIIC/TFIIIB complex on tRNA genes and the average distance of approximately 50 bp of TRE5-A integrants to tRNA genes (Fig. 2), it is possible that the TRE5-A pre-integration complex targets TFIIIB subunits. More study is required to determine the integration preference of *de novo* TRE integrants in the presence or absence of pol III transcription factors bound to target tRNA genes. Studies to address this question have been recently performed and will be described below.

3.4. Assays to test the TRE5-A integration specificity in vivo

The first *Dictyostelium in vivo* retrotransposition assay was developed in Theo Dingermann’s lab and is referred to as the “TRE trap” (Beck et al. 2002; Siol et al. 2006). The TRE trap uses a modified *D. discoideum* UMP synthase gene, which is cloned into plasmids and is transformed into uracil-auxotrophic mutants (Fig. 3A). Complementation with the plasmid-encoded UMP synthase gene converts transformants from a ura^- to a ura^+ phenotype. If a tRNA gene (the bait) is cloned into the intron of the UMP synthase gene, transformants carrying the TRE trap will have a ura^+ phenotype and are sensitive to the cytostatic drug 5-fluoroorotic acid (5-FOA). Cells bearing the TRE trap may acquire mutations of the artificial UMP synthase gene (the TRE trap) by integration of TRE5-A upstream of the bait tRNA gene. Integration events would thus convert affected cells to the ura^- phenotype, conferring resistance to 5-FOA that allows them to grow clonally under selection in 5-FOA when fed uracil (Fig. 3A).

Using the TRE trap assay, we measured the activity of the natural population of TRE5-A elements in the *D. discoideum* genome. The strength of the assay is that it makes use of a defined integration target site that can be easily analyzed. For instance, it is possible to determine how frequently TRE5-A elements integrate into the TRE trap if the sequence of the bait tRNA gene is manipulated to prevent the binding of the transcription factor TFIIIC (Siol et al. 2006). Such analyses have shown that the binding of TFIIIC to a tRNA gene is a prerequisite for TRE5-A integration. Recent *in vitro* data suggest that TFIIIB subunits are involved in protein-protein interactions with the TRE5-A-encoded ORF1 protein (Chung et al. 2007). Thus, the current model predicts that TFIIIC binding to tRNA genes is required to recruit TFIIIB to the genomic DNA and that protein-protein interactions of TFIIIB with the TRE5-A ORF1 protein determine integration sites.

As the TRE trap assay relies on one particular integration target, it is principally unsuitable to address the question of the *a priori* integration preference of TRE5-A. In other words, the question of how often mobile TRE5-A elements would

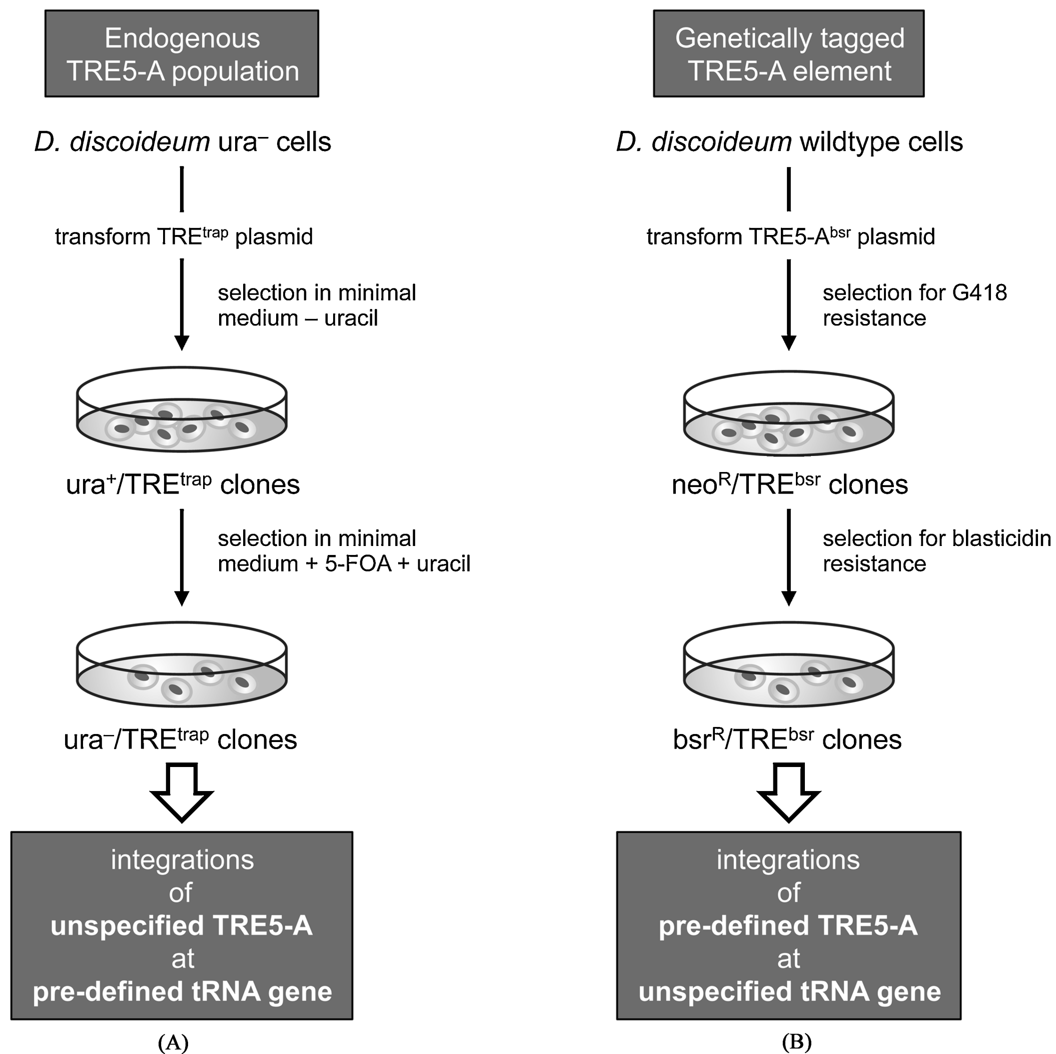

Fig. 3: *In vivo* assays to study TRE5-A retrotransposition. (A) The TRE trap assay determines the retrotransposition activity of the cellular TRE5-A population. A defined tRNA gene is cloned into the intron of a plasmid-borne UMP synthase gene to generate the TRE trap (see Beck et al. 2002 for details). The TRE^{trap} plasmid is transformed into ura^- cells, and complementation is selected for by culturing the cells in the absence of uracil. Resulting ura^+ cells are susceptible to killing with 5-fluoroorotic acid (5-FOA). Integration of TRE5-A into the TRE trap converts the cells back to ura^-. Such cells gain 5-FOA resistance and can grow in medium containing uracil. Note that the integration of natural TRE5-As at the pre-defined integration site allows for the analysis of structural requirements of genomic loci to be recognized as integration sites by TRE5-A. (B) Retrotransposition assay using genetically tagged TRE5-A retrotransposons. TRE5-A^{bsr} elements carry a blasticidin resistance gene that is specifically activated only if a complete cycle of transcription of TRE5-A^{bsr}, reverse transcription, and integration has occurred (see Siol et al. 2011 for details). The TRE5-A^{bsr} is transformed into *D. discoideum* cells, and transformants expressing the TRE5-A^{bsr} "master element" are selected by culturing cells in G418 (selecting for neo^R). Each retrotransposition event of a master element will render the cells resistant to blasticidin. Note that in this assay, the TRE5-A^{bsr} elements mimic the natural integration preference of natural TRE5-As; thus, it is possible to identify natural integration sites in a genome-wide approach, and the assay can be used to determine the rate of TRE5-A off-site integrations

choose "off-target" integration sites and thus be mutagenic remains unanswered. To approach this interesting question, we have recently designed genetically tagged TRE5-A retrotransposons (Fig. 3B) (Siol et al. 2011). These elements are stably integrated into the *D. discoideum* genome by transformation and are referred to as the "master elements" from which copies can be produced by transcription, reverse transcription, and integration at any genomic position naturally accessible by TRE5-A retrotransposons. The master element carries a non-functional blasticidin resistance cassette that becomes active after one complete retrotransposition cycle (Fig. 3B). Thus, any retrotransposed TRE5-A copy will render its host cell blasticidin-resistant, and clones of such cells can be analyzed for integration sites in genome-wide settings.

In the future, genetically tagged TRE5-A retrotransposons will help to answer the fundamental question of whether the tRNA gene preference of TRE5-A elements is as strict as predicted by Dingermann and co-workers some 20 years ago from the analysis of "old" genomic insertions. This information in turn will be critically important in determining whether the exceptional target site preference of TRE5-A integration might present a non-human model system to address a major drawback in the design of integrating gene transfer vectors in human gene therapy: their poor target site preference.

3.5. The dark side of untargeted vector integration in gene therapy

The term "gene therapy" is commonly used to describe the transfer of functional genes into somatic cells of patients suffering from disease caused by a particular gene defect. The clinical success of this approach depends on several variables such as efficacy of gene transfer and the maintenance of transgene expression in the genetically modified cells. Retroviral vector systems have been widely utilized for clinical gene transfer because they possess a highly efficient, enzyme-catalyzed integration step that will stably fix the transgene in the genome of modified patient cells (Naldini 2011). Chromosomal integration

is often associated with long-lasting expression of the transgene and sufficient phenotype correction.

Gene therapy using vectors derived from simple γ-retroviruses have been widely applied, especially in early clinical trials, and have been particularly successful for the treatment of life-threatening severe combined immunodeficiency (SCID) caused by the lack of either adenosine deaminase (ADA-SCID) or the interleukin-2 receptor common gamma chain (SCID-X1) (Fischer et al. 2010). In five clinical trials, 27 patients suffering from ADA deficiency and 20 patients with SCID-X1 were treated in the period of 1999–2009. Although most of the treated patients did significantly benefit from the gene therapies, five cases of clonal T cell proliferation occurred between 23 and 68 months after gene therapy (Fischer et al. 2010). Retrospective analyses revealed that the observed T cell leukemias were directly associated with integration of the γ-retroviral gene transfer vectors at harmful sites in the patient's genomes. The gene transfer vectors frequently inserted in the vicinity of proto-oncogenes such as LMO2 and upregulated their expression due to the activity of strong promoter/enhancer functions present in the γ-retroviral LTRs. This resulted in dominant gain-of-function mutations causing a selective advantage of the engrafted cells compared with other hematopoietic progenitor cells, leading to the observed clonal T-cell proliferation (Biasco et al. 2012).

The clinical trials for SCID treatment accentuated the problem of insertional genotoxicity associated with γ-retroviral gene therapy vectors. Studies with animal models (Wu et al. 2003) and analysis of bone marrow of patients enrolled in gene therapy trials (Hacein-Bey-Abina et al. 2008, 2003; Howe et al. 2008; Stein et al. 2010) have revealed that γ-retroviral vectors do not integrate randomly but have a strong trend to accumulate close to transcription start sites and regulatory regions. Even worse, γ-retroviral vectors have been shown to prefer integration sites near genes involved in the control of cell proliferation (Cattoglio et al. 2007).

Several improvements have been implemented in second generation retroviral gene transfer vectors in order to reduce insertional genotoxicity (Baum et al. 2011; Kustikova et al. 2010; Modlich and Baum 2009). Most notable are lentiviral vectors derived from human immunodeficiency virus-1 (HIV-1). Lentiviral vectors are considered to be superior to γ-retroviral vectors because they are capable of infecting resting cells, whereas γ-retroviral gene transfer is possible only in proliferating cells. Further, lentiviral vectors integrate preferentially into actively transcribed genes (Schröder et al. 2002), but they are much less biased to promoter regions and provoke fewer effects on the expression of nearby (onco)genes. Another improvement to the safety of retroviral vectors was the introduction of self-inactivating retroviral (SIN) LTRs (Yu et al. 1986). SIN LTRs are modified to delete the promoter/enhancer region from the LTR, such that integrated gene transfer vectors no longer affect the expression of neighboring genes. As a consequence, the expression of the therapeutic transgene is no longer driven from the left LTR as in the first-generation γ-retroviral vectors but instead from internal heterologous promoters.

Combining the use of lentiviral vectors and SIN LTRs is considered a vast improvement for the safety of retroviral gene transfer systems, but the concern regarding insertional gene disruption remains. It can be argued that this is not a concern in the clinic because in most cases, only one allele is altered and a heterozygous loss-of-function mutation may not cause a phenotype. However, cell physiology can be altered by insertional mutagenesis if a single intact allele of a certain gene causes haploinsufficiency or if genes that control cell growth and/or differentiation are affected. Gene transfer vector targeting to "safe" places in the genome would significantly reduce genotoxicity, thereby illuminating the "dark side of untargeted vector integration" (Kustikova et al. 2010) in gene therapy.

A promising aspect of targeted gene delivery into human cells is the use of transposons as gene shuttles (Di Matteo et al. 2012). Retroviruses are simply "infectious mobile elements". Thus, nonviral transposable elements can be considered suitable gene shuttles even though they must be introduced into patient cells by chemical or physical methods that are less efficient compared to the natural infection by retroviruses. As there are no known site-specific integrating retroviruses, transposable elements that possess natural target site selectivity would be attractive for the development of gene transfer vectors. Ivicz and coworkers have successfully applied the transposon Sleeping Beauty (SB) to clinical practice (Swierczek et al. 2012). SB is highly active in human cells, but it has no intrinsic target site selectivity. It is nevertheless considered a safer gene transfer system because it lacks the retroviral preference for transcribed regions of the genome. Recent improvements of SB-derived gene delivery systems include the coupling of SB transposase to zinc finger nucleases (ZFN), which are DNA sequence-specific endonucleases that can be designed to recognize and cleave virtually any user-defined DNA target. ZFN-directed SB is capable of delivering transgenes with nearly 50% frequency to desired positions in the human genome (Voigt et al. 2012).

4. Conclusion

"An added value from the studies of simple models is that, not infrequently, they open radically new perspectives in the study of a given pathology" (Escalante 2011).

One of the major drawbacks in the development of gene therapy to clinical application is the putative genotoxicity of retroviral gene transfer vectors. As there are no natural prototypes of site-specific integrating retroviruses, combining powerful genetic modification of cells through infection by retroviruses with highly specific targeting mechanisms of transposons such as the DRE element may pave the way to next-generation gene transfer systems with low intrinsic genotoxicity. Current research on the *Dictyostelium* TRE5-A retrotransposon is focused on elucidating the degree of targeting efficacy and the protein-protein interactions required for tRNA gene recognition. Some 25 years after the discovery of the TRE5-A element, it still remains to be seen whether a TRE5-A-derived tRNA gene targeting approach in human cells can reduce the inherent genotoxicity of retroviral vectors.

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