CellPress
REVIEWS

Spotlight

Prime Editing: A New Way for Genome Editing

Marek Marzec,[1,*] Agnieszka Brąszewska-Zalewska,[1] and Goetz Hensel[2,3]

Precise and efficient use of genome editing tools are hampered by the introduction of DNA double-strand breaks, donor DNA templates, or homology-directed repair. A recent study expands the genome editing toolbox with the introduction of prime editing, which overcomes previous challenges and introduces insertions, deletions, and all putative 12 types of base-to-base conversions in human cells.

Due to the increasing availability of genomic sequences for many species and their usage for basic as well as applied research, a tool that allows specific modifications of a certain gene is needed. The past two decades have brought great progress in developing tools for targeted genome modification [i.e., the zinc-finger nucleases (ZFNs) [1] and transcription activator-like effector nucleases (TALENs)] [2] that are able to cleave double-stranded DNA at a specific region. However, both techniques remain expensive and the generation of proteins capable of binding targeted DNA is a time-consuming process. RNA-guided endonucleases have also been used for genome modification. Using two components of the clustered regularly interspaced short palindromic repeats and CRISPR-associated (CRISPR/Cas) prokaryote immune system, it was possible to introduce double-stranded breaks in target DNA [3]. In this system, the Cas protein (i.e., Cas9) is guided by short RNA sequence (sgRNA), to the specific region of genome that is cleaved within both DNA strands. This is possible because of the presence of 18–22 nucleotides (called spacer) in sgRNA sequence that are complementary to the targeted DNA. When this break is repaired via the preferential nonhomologous end-joining DNA repair pathway, random insertions or deletions (indels) are introduced in the target sequence(s) [3]. Discovery of novel Cas endonucleases and the development of mutated versions of known Cas endonucleases has increased the specificity and efficiency of these techniques [4]. Another breakthrough in genome editing was the utilization of RNA-guided endonucleases for base editing, including all four transitions: C→T, T→C, A→G, and G→A [5].

A major limitation of the current genome editing technologies has been the ability to provide the altered customized sequence simultaneously at the target site. Recently, a method to overcome such challenges, known as prime editing, has been described by Anzalone *et al.* in *Nature*. Prime editing enables the introduction of indels and all 12 base-to-base conversions (both transitions and transversions) without inducing a DNA double-strand break [6]. Here, a prime editing guide RNA (pegRNA) drives the Cas9 endonuclease. Moreover, the pegRNA contains not only the spacer that is complementary to one DNA strand but also a primer binding site (PBS) region and the sequence that will be introduced to the targeted gene. The PBS region is complementary to the second DNA strand and will create a primer for the reverse transcriptase (RT) that is linked to the Cas9(H840A) nickase. The RT is an RNA-dependent DNA polymerase that uses the sequence from the pegRNA as a template. Then the information is copied directly from the pegRNA into target DNA sequence; therefore, altering the preselected target sequence in a customized manner. After this step, two redundant single-stranded DNA flaps remain: original, unmodified DNA (5′ flap) and edited DNA that was copied from pegRNA (3′ flap) [6] (Figure 1). These overhangs are subsequently stabilized and integrated into the genome via a DNA repair system present in cells.

Three generations of prime editors (PEs) have been successfully tested in human cells [6]. In the first generation of PEs (PE1), Moloney murine leukemia virus reverse transcriptase (M-MLV RT), which is an RNA-dependent DNA polymerase, was linked to the C terminus of Cas9 nickase (H840A), which is an endonuclease with one inactivated domain. This complex was driven by pegRNA expressed in the second plasmid. The pegRNA contains from eight to 15 bases of PBS, template sequence for RT (containing the mutation that will be introduced), and the spacer sequence that will bind the target DNA. The efficiency of PE1 reached values of 0.7–5.5% in the case of introduction of point transversions. The efficiency depends on the PBS length and, for different genes, the varied length of PBS (from eight to 16 nucleotides) results in the highest efficiency [6].

To improve efficiency of the PE, different variants of M-MLV RT, which contain mutations affecting thermostability, processivity, DNA–RNA substrate affinity, and RNaseH activity were used. Introduction of three mutations (D200N, L603W, T330P), which increased RT activity at elevated temperatures, also increased the number of introduced transversions (up to 6.8-fold in comparison with nonmutated RT). Two additional mutations (T306K, W313F) that enhance binding of RT to the template–PBS complex and RT thermostability, improved editing efficiency (1.3–3.0-fold). Finally, pentamutant RT linked to the nickase [Cas9(H840A)-M-MLV RT (D200N/L603W/T330P/T306K/W313F)] was described as a second generation PE (PE2), which exhibits 1.6–5.1-fold improvement in efficiency of introducing point mutations, when compared with PE1.

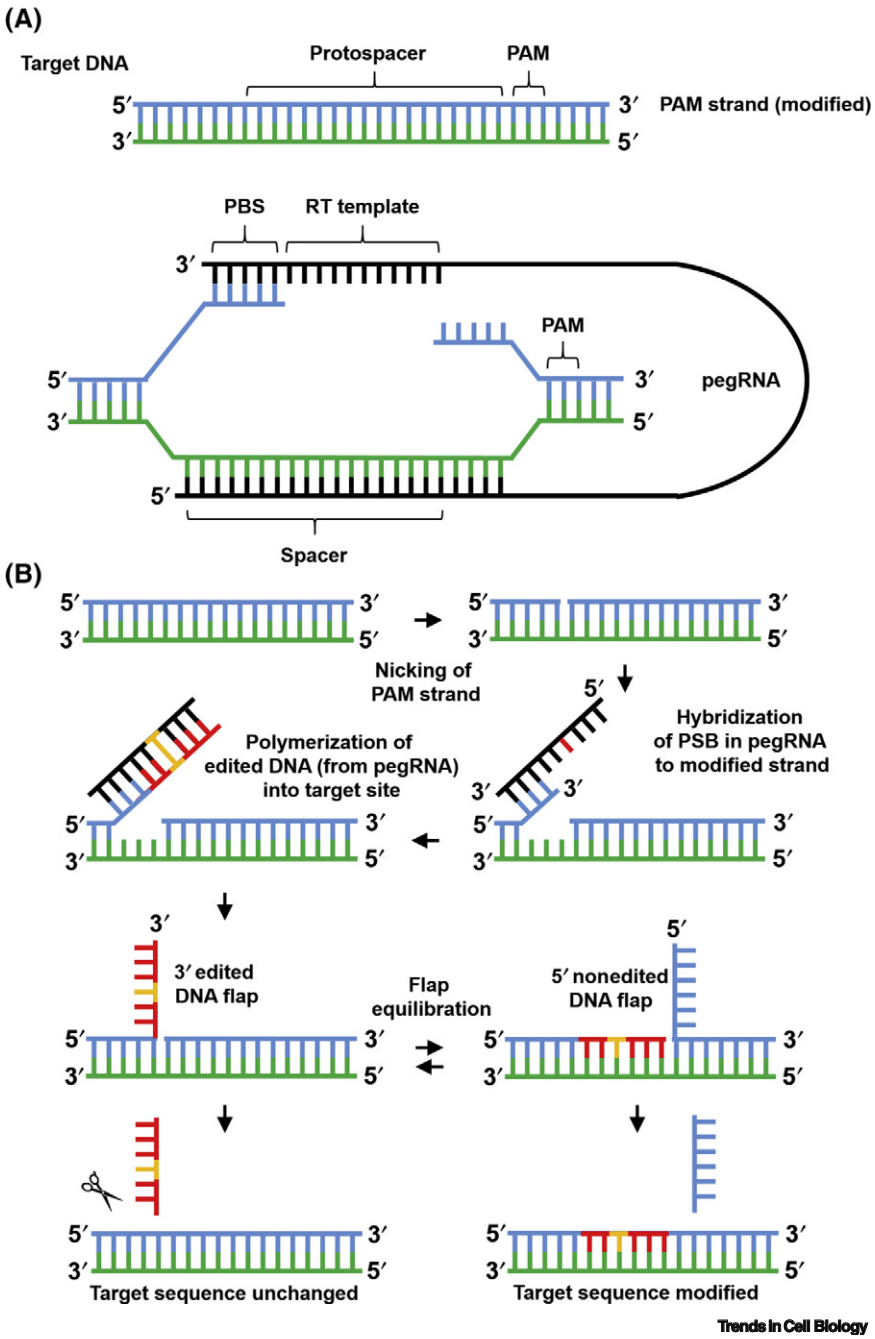

Figure 1. Prime Editing System. (A) Hybridization between prime editing guide RNA (pegRNA) and target DNA. Spacer of pegRNA recognize sequence of non-protospacer adjacent motif (PAM) DNA strand, whereas primer binding site (PBS) of pegRNA recognize sequence of PAM DNA strand. (B) Mechanism of prime editing. PAM strand is nicked by Cas9(H840A) nickase and hybridization between PBS and PAM DNA strand occurs. Reverse transcriptase (RT) copy information from pegRNA into 5′ DNA flap. Next, there are two possibilities: (i) flap equilibration results in hybridization of 5′ DNA flap to DNA unmodified strand and DNA repair process may introduce mutation to the second DNA strand; or (ii) flap equilibration does not occur, 5′ DNA flap is excised, and target sequence remains unchanged.

In the present system, there are only two factors that limit the efficiency of DNA edits: the choice of which single-stranded DNA flaps (edited or nonedited) will be paired with unmodified DNA strand and which DNA strand (edited or unmodified) will be used as a template during DNA repair. It was shown that a nick in the unmodified strand increased efficiency of the base editing systems in animal and plant cells [7–10]. To apply this strategy to PE, nickase was used that was guided by the classical sgRNA with the spacer matching only the edited sequence. This approach, called PE3 system, increased the efficiency (threefold) in introducing point mutations [6]. Finally, all 12 possible transition and transversion mutations were generated with 33% (±7.9%) efficiency in the PE3 system, which is similar to the efficiency level of existing systems of base editing (cytidine and adenine base editors). However, the number of off-target effects observed for PEs was reduced when compared with Cas9, even when the same protospacer was used. In human cells, only three of 16 known Cas9 off-target sites for *HEK3*, *HEK4*, *EMX1*, and *FANFC* loci were modified by PE3 [6]. This increased specificity is due to the three steps of DNA hybridization that are present during prime editing events: between target DNA and spacer from pegRNA; between target DNA and PBS from pegRNA; and between target DNA and edited DNA flap. In the case of the standard Cas9 system, only hybridization between target DNA and protospacer from sgRNA occurs.

The prime editing system is a milestone in the development of a precise and universal method for genome editing. It needs to be highlighted that for the first time all 12 point mutations can be introduced to target genes at locations ranging from 3 bp upstream to 29 bp downstream of a protospacer adjacent motif. However, PEs were also used to perform insertions even up to 44 bp and deletions up to 80 bp [6]. Further optimization of PEs would increase their efficiency and

specificity, features that are crucial for their medical use. Adaptation of PE systems for other animal cells, as well as plant genomes, will give researchers a new tool for basic studies of gene functions, with previously unachievable possibilities.

[1]University of Silesia in Katowice, Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, 40-032 Katowice, Poland
[2]Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany
[3]Division of Molecular Biology, Centre of the Region Hana for Biotechnological and Agriculture Research, Faculty of Science, Palacký University, Olomouc, Czech Republic

*Correspondence:
marek.marzec@us.edu.pl (M. Marzec).

https://doi.org/10.1016/j.tcb.2020.01.004

References

1. Urnov, F.D. *et al.* (2010) Genome editing with engineered zinc finger nucleases. *Nat. Rev. Genet.* 11, 636
2. Joung, J.K. and Sander, J.D. (2013) TALENs: a widely applicable technology for targeted genome editing. *Nat. Rev. Mol. Cell Biol.* 14, 49
3. Jinek, M. *et al.* (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821
4. Marzec, M. and Hensel, G. (2019) More precise, more universal and more specific–the next generation of RNA-guided endonucleases for genome editing. *FEBS J.* 286, 4657–4660
5. Rees, H.A. and Liu, D.R. (2018) Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* 19, 770–788
6. Anzalone, A.V. *et al.* (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 576, 149–157
7. Komor, A.C. *et al.* (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533, 420
8. Gaudelli, N.M. *et al.* (2017) Programmable base editing of A• T to G• C in genomic DNA without DNA cleavage. *Nature* 551, 464
9. Zong, Y. *et al.* (2017) Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat. Biotechnol.* 35, 438
10. Lu, Y. and Zhu, J.K. (2017) Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. *Mol. Plant* 10, 523–525

Spotlight

Multiciliated Cells: Rise and Fall of the Deuterosomes

Dheeraj Rayamajhi[1,2] and Sudipto Roy[1,2,3,*]

Esoteric organelles called deuterosomes have been implicated in the explosive production of hundreds of basal bodies in multiciliated cells (MCCs). A new study by Meunier, Holland, and colleagues now shows that deuterosomes are dispensable, re-igniting the quest for mechanisms driving basal body biogenesis in this specialized ciliated cell type.

MCCs, with their apical arrays of motile cilia, drive fluid movement over epithelia [1]. For example, MCCs within the airways clear mucus and those lining the ependyma of the brain ventricles facilitate cerebrospinal fluid flow. Formation of multiple motile cilia requires a large number of centrioles to be first generated in postmitotic MCC precursors, which later convert into ciliary basal bodies (Figure 1A). Studies over the years have postulated two pathways for centriole amplification in MCCs: the centriole-dependent (CD) and deuterosome-dependent (DD) pathways. As the name suggests, the first pathway involves centrosomal (parental) centrioles for templating procentriole biogenesis. The second pathway is thought to rely on abstruse electron-dense fibrogranular ring-like structures termed deuterosomes. Discovered through electron microscopic studies in the late 1960s and early 1970s [2], deuterosomes are unique to MCCs, and arise during the early centriole amplification stage, to disappear once amplification is completed. The DD pathway is considered to be the major contributor of centriole production in MCCs; about 90% of centrioles in mammalian MCCs are currently believed to arise via the DD pathway.

Despite the purported importance of the deuterosomes, there has been an ongoing controversy regarding their origins. Some researchers argue that the organelles are derived from parental centrioles, while others consider a *de novo* origin. Recent findings of Zhao *et al.* from the Zhu laboratory support the second theory [3]. Inhibition of Plk4 (a kinase essential for parental centriole duplication in cycling cells) in cultured mouse ependymal cells (mEPCs), which rendered them devoid of the parental centrioles, did not derail deuterosome formation or multiple basal body production (Figure 1B). The authors also demonstrated that under such circumstances, deuterosomes were the nucleation centers for nascent procentrioles. Similar observations were made in two other independent studies [4,5], providing strong evidence in support of the view that MCCs can produce basal bodies without parental centrioles, via the DD pathway. With centrioles being dispensable, the role of the DD pathway in MCC basal body biogenesis assumed an even greater significance.

However, the latest report, that of Mercey *et al.* published in *Nature Cell Biology*, delivers a fatal blow to the deuterosomes [6]. This work, a collaborative effort from the Meunier and Holland laboratories, shows that MCCs can generate the correct numbers of basal bodies without deuterosomes, and more surprisingly, even in the combined absence of deuterosomes and parental centrioles, and tentatively implicate the pericentriolar cloud as the source of procentriole assembly.

Earlier pioneering work from the Zhu laboratory identified Deup1 (deuterosomal protein 1), as a paralog of the centriolar protein Cep63, that is expressed specifically in differentiating MCCs. They showed that Deup1 is a deuterosome-specific structural protein; the first such molecular component of the deuterosome to be characterized, and using knockdown strategies in cultured mouse tracheal MCCs (mTECS) and *Xenopus* embryos (which differentiate MCCs on their skin), they found it to be a critical determinant