

Meganuclease: from DNA Double-Strand Breaks to Gene Therapy

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Meganucleases are sequence specific endonucleases with large recognition sites, which can stimulate homologous gene targeting up to 10,000-fold (Rouet, Smih et al. 1994; Choulika, Perrin et al. 1995; Puchta, Dujon et al. 1996; Sargent, Brenneman et al. 1997; Donoho, Jasin et al. 1998; Elliott, Richardson et al. 1998). These findings are at the basis of the concept of genome surgery, the efficient and precise editing of genomes in living organisms. Thus, meganucleases may represent a universal tool for genome engineering, including the correction of mutations involved in monogenic inherited diseases. Genome surgery aims at the correction of the endogenous gene, and has therefore tremendous advantages. It is not associated with the limitations of the classical gene therapy complementation strategy (Kohn, Sadelain et al. 2003; Cavazzana-Calvo, Lagresle et al. 2005; Fischer, Le Deist et al. 2005), such as deleterious random insertion and short duration of transgene expression. Now, therapeutic applications depend on our ability (i) to develop novel meganucleases with dedicated specificities and (ii) to use meganucleases to trigger gene correction in stem cells or somatic tissues, with minimal toxicity.

The generation of novel meganucleases with tailored specificities is under intense investigation. Recently, fusion of Cys2-His2 type Zinc-Finger Proteins (ZFP) with the catalytic domain of the FokI nuclease were used to make functional sequence-specific endonucleases (Smith, Berg et al. 1999; Urnov, Miller et al. 2005). The binding specificity of ZFPs is relatively easy to manipulate, and a repertoire of novel artificial ZFPs, able to bind many (G/A)NN(G/A)NN(G/A)NN sequences is now available (Isalan, Klug et al. 2001; Pabo, Peisach et al. 2001; Segal and Barbas 2001). Nevertheless, preserving a very narrow specificity is one of the major issues for genome engineering applications, and presently it is unclear whether ZFPs would fulfill the very strict requirements for therapeutic applications.

Homing Endonucleases (HEs) are a widespread family of natural meganucleases including hundreds of proteins (Chevalier and Stoddard 2001). These proteins are encoded by mobile genetic elements which propagate by a process called "homing": the endonuclease cleaves a cognate allele from which the mobile element is absent, thereby stimulating a homologous recombination event that duplicates the mobile DNA into the recipient locus. Given their natural function and their exceptional specificity, HEs provide ideal scaffolds to derive novel endonucleases for genome engineering. Data have been accumulated over the last decade, characterizing the LAGLIDADG family, the largest of the four HE families (Chevalier and Stoddard 2001). Seven different LAGLIDADG proteins have been crystallized, and they exhibit a very striking conservation of the core structure (Chevalier, Monnat et al. 2001; Chevalier and Stoddard 2001; Moure, Gimble et al. 2002; Bolduc, Spiegel et al. 2003; Chevalier, Turmel et al. 2003; Moure, Gimble et al. 2003). These structural similarities prompted the construction of chimeric and single chain artificial HEs (Chevalier, Kortemme et al. 2002; Epinat, Arnould et al. 2003; Steuer, Pingoud et al. 2004), showing that these proteins were robust enough to withstand extensive modifications. We have used a rational approach along with a cell based high-throughput assay that directly monitors endonuclease-induced recombination in living cells, to identify functional variants of the I-Crel homodimeric protein. Hundreds of homing endonucleases with new specificities were identified, and stored into our database (Omegabase). Many new proteins maintained their very narrow specificity and displayed high levels of cleavage on new targets in living cells, showing that engineering can preserve efficacy and specificity. The modular structure

of the protein allows for the combination of two different LAGLIDADG domains. In addition, smaller independent subdomains can be identified, so we can combine up to four different meganuclease variants into novel functional proteins with predictable specificities. Together with the collection of large samples of novel homing endonuclease variants, this combinatorial approach results in an exponentially growing number of functional meganucleases, giving the possibility to cleave natural sequences.

With these novel proteins, therapeutical applications such as the correction of mutations involved in monogenic inherited diseases can be envisioned. However, efficacy and toxicity have to be evaluated. Using a mouse model, we have investigated the use of meganuclease to induce targeted homologous recombination in living animal. One major finding is that meganuclease-induced recombination could be achieved in an adult organ. It was not clear whether adult liver somatic cells would be proficient for homologous recombination. Our results reveal that meganucleases can also be used to induce homologous recombination, at least between direct repeats, in hepatocytes *in toto*. The relatively high frequency of recombination in liver (1.3%) is similar to what we found in cellular models using the same constructs. In the future, liver monogenetic diseases could then be among the principal targets to apply this genome engineering technology in the field of gene therapy. In the present study, the vectorisation technique used limited our observation to this individual organ; however, other methods should allow us to extend this survey to other tissues. Altogether, our results open the door for a broad range of *in toto* applications although the potential toxicity of meganucleases revealed by lethality after high dose injection will need to be further investigated.

About Collectis

Collectis SA was founded in 1999, as a spin-off from the Institut Pasteur. The company is developing enabling technology for commercial applications as human therapeutics for genetic and infectious diseases, pharmaceutical discovery, agriculture and industrial biotechnology. The technological breakthrough is the Meganuclease Recombination Systems (MRS©). Based on a novel class of genetic scissors (the Meganucleases), this technology performs precise modifications of cellular chromosomes. Unlike alternative technologies, the Meganuclease Recombination System (MRS©) makes a physical correction of genetic errors by genuine homologous recombination. Collectis aims at efficiency and precision in order to make genome reprogramming accessible to the biotechnology and pharmaceutical industry. Collectis has developed specific skills in the field of genome engineering and protein engineering. One of the great achievements of the company is the set up of a High-Throughput Screening platform. Based on a cell-based functional assay for the identification of novel endonucleases, this platform has delivered hundreds of novel meganucleases. As of today, Collectis has 33 employees, including 13 PhDs, and has signed more than 30 deals on its genome engineering technologies with major AgroSciences and world leading biotechnology companies, among which stand Biogen Idec, Merck, DuPont/Pioneer, BASF Plant Science, Limagrain Group, Diversa and Regeneron Pharmaceuticals.

References

- Bolduc, J. M., P. C. Spiegel, et al. (2003). "Structural and biochemical analyses of DNA and RNA binding by a bifunctional homing endonuclease and group I intron splicing factor." *Genes Dev* **17**(23): 2875-88.
- Cavazzana-Calvo, M., C. Lagresle, et al. (2005). "Gene therapy for severe combined immunodeficiency." *Annu Rev Med* **56**: 585-602.
- Chevalier, B., M. Turmel, et al. (2003). "Flexible DNA target site recognition by divergent homing endonuclease isoschizomers I-Crel and I-Msol." *J Mol Biol* **329**(2): 253-69.
- Chevalier, B. S., T. Kortemme, et al. (2002). "Design, activity, and structure of a highly specific artificial endonuclease." *Mol Cell* **10**(4): 895-905.
- Chevalier, B. S., R. J. Monnat, Jr., et al. (2001). "The homing endonuclease I-Crel uses three metals, one of which is shared between the two active sites." *Nat Struct Biol* **8**(4): 312-6.
- Chevalier, B. S. and B. L. Stoddard (2001). "Homing endonucleases: structural and functional insight into the catalysts of intron/intein mobility." *Nucleic Acids Res* **29**(18): 3757-74.
- Choulika, A., A. Perrin, et al. (1995). "Induction of homologous recombination in mammalian chromosomes by using the I-SceI system of *Saccharomyces cerevisiae*." *Mol Cell Biol* **15**(4): 1968-73.
- Donoho, G., M. Jasin, et al. (1998). "Analysis of gene targeting and intrachromosomal homologous recombination stimulated by genomic double-strand breaks in mouse embryonic stem cells." *Mol Cell Biol* **18**(7): 4070-8.
- Elliott, B., C. Richardson, et al. (1998). "Gene conversion tracts from double-strand break repair in mammalian cells." *Mol Cell Biol* **18**(1): 93-101.
- Epinat, J. C., S. Arnould, et al. (2003). "A novel engineered meganuclease induces homologous recombination in yeast and mammalian cells." *Nucleic Acids Res* **31**(11): 2952-62.
- Fischer, A., F. Le Deist, et al. (2005). "Severe combined immunodeficiency. A model disease for molecular immunology and therapy." *Immunol Rev* **203**: 98-109.
- Isalan, M., A. Klug, et al. (2001). "A rapid, generally applicable method to engineer zinc fingers illustrated by targeting the HIV-1 promoter." *Nat Biotechnol* **19**(7): 656-60.
- Kohn, D. B., M. Sadelain, et al. (2003). "Occurrence of leukaemia following gene therapy of X-linked SCID." *Nat Rev Cancer* **3**(7): 477-88.
- Moure, C. M., F. S. Gimble, et al. (2002). "Crystal structure of the intein homing endonuclease PI-SceI bound to its recognition sequence." *Nat Struct Biol* **9**(10): 764-70.
- Moure, C. M., F. S. Gimble, et al. (2003). "The crystal structure of the gene targeting homing endonuclease I-SceI reveals the origins of its target site specificity." *J Mol Biol* **334**(4): 685-95.
- Pabo, C. O., E. Peisach, et al. (2001). "Design and selection of novel Cys2His2 zinc finger proteins." *Annu Rev Biochem* **70**: 313-40.
- Puchta, H., B. Dujon, et al. (1996). "Two different but related mechanisms are used in plants for the repair of genomic double-strand breaks by homologous recombination." *Proc Natl Acad Sci U S A* **93**(10): 5055-60.
- Rouet, P., F. Smih, et al. (1994). "Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease." *Mol Cell Biol* **14**(12): 8096-106.
- Sargent, R. G., M. A. Brennehan, et al. (1997). "Repair of site-specific double-strand breaks in a mammalian chromosome by homologous and illegitimate recombination." *Mol Cell Biol* **17**(1): 267-77.
- Segal, D. J. and C. F. Barbas, 3rd (2001). "Custom DNA-binding proteins come of age: polydactyl zinc-finger proteins." *Curr Opin Biotechnol* **12**(6): 632-7.
- Smith, J., J. M. Berg, et al. (1999). "A detailed study of the substrate specificity of a chimeric restriction enzyme." *Nucleic Acids Res* **27**(2): 674-81.
- Steuer, S., V. Pingoud, et al. (2004). "Chimeras of the homing endonuclease PI-SceI and the homologous *Candida tropicalis* intein: a study to explore the possibility of exchanging DNA-binding modules to obtain highly specific endonucleases with altered specificity." *ChemBiochem* **5**(2): 206-13.
- Urnov, F. D., J. C. Miller, et al. (2005). "Highly efficient endogenous human gene correction using designed zinc-finger nucleases." *Nature*.