RESEARCH PAPER | Science | Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

High Efficiency Genetic Modification Tools for Site Specific Recombination

KEYWORDS: Site Specific Recombination, Target sites, Genetic Recombination

Priyadarshini K	Bańcyr Edyta	Żyromsk Marcin
Department of Biotechnology, JSS College for Arts Commerce and Science, Mysore, India	Department of Veterinary Medicine, Wroclaw University of Environmental and Life Science, Poland	Department of Chemistry, Wroclaw University of Environmental and Life Science, Polands

ABSTRACT *Genetic engineering has revolutionized our current understanding of genetics. Importantly, genetic engineering has become a tool used to defeat many genetic diseases. Correcting congenital or acquired genetic errors in medicine, as well as introducing desired genotypes in pharmaceutical and agricultural biotechnology require genetic engineering. In this work, we review different techniques based on genetic recombination. In particular, we outline the principles of site specific recombination and transpositional site specific recombination, Cre-lox system of recombination, Zinc finger technique FLP-FRT, and Crisp. We discuss various approaches of targeting the selected genes.*

INTRODUCTION

HISTORIC OVERVIEW

Evolutionary changes, following over a billion years of life on Earth, contributed to the emergence of many new traits in organisms. Genetic recombination is considered to be a key determinant of the process of evolutionary change, providing the so-called "evolutionary dynamics of genome" affecting the generation of new combinations of alleles, maintaining genome stability and also in maintaining telomere.

CONSERVATIVE SITE SPECIFIC RECOMBINATION

In our review, we try describe important for scientist site specific recombination technologies and their applications. What is more, we focus on a few important for us technologies, like cre-lox system of recombination, cre recombinase, FRT, genetic recombination, homologous recombination, aptamer-guided gene targeting, and zinc finger recmobinase system. We try to show, why new technologies are important for scientist to understand evolution.

CRE-LOX SYSTEM OF RECOMBINATION

The mechanism was described as a part of viral life cycle, and the mechanism was discovered in P1 bacteriophage (Sternberg and Hamilton 1981). Mechanism of cre-lox recombination has been developed as an important technology for genome manipulation, and it was applied in different cell's cultures, yeast, plants, and in organisms.

Cre-lox system has got two components: special enzyme-Cre recombinase- which catalyzes recombination between both sites of loxP. Cre- gene is a short name for cyclization recombination. Cre protein is useful to recombine DNA, when it locates specific sites in DNA molecule. These special sites are known as a lox P sites. Lox P (locus of X-over P1) sites are described as a 34-base pair sequences, complex of 8bp core sequence which confers directionality, and two flaking 13bp repeats (Metzger and Feil 1999). LoxP sequences are isolated from bacteriophage P1, and these sequences are not found in animal or plant cells. Cre-recombinase is counted to type I isomerase, isolated from bacteriophage P1. Interestingly, cre-recombinase requires no cofactors energy. Crystallographic studies by the groups of Van Duyne and Baldvin has shed light on mechanism of interactions between Cre-recombinase and DNA (Ennifar et al. 2003). Cre recmobinase can bind to recogniton elements, and forming C-shaped clamp, around the DNA duplex. Important role during genetic recombination is playing Holliday junctions (HJ), mobile junctions between four strands of DNA. The structure determination of a Cre–Holliday junction complex has provided an indication of the nature of this isomerization step, at least for the Cre–loxP pathway. In the offspring, of restricted DNA fragment tested loxP sites, is thus cut on only tissue where Cre recombinase recombination occurs (Górska and Kowalski 1997).

ZINC FINGER TECHNIQUE

The serine recombinases are a miscellaneous family of modular enzymes (Prorocic et al. 2011), tightly controlled by intricate, topologically-defined, nucleoprotein complexes that promote high-fidelity DNA rearrangements among specific target sites (Wu et al. 1993). Engineered zinc-finger recombinases (ZFRs) are produced by replacement of their local DNA-binding domains with custom-designed Cys2–His2 zinc-finger proteins that could be capable of achieving targeted genetic modifications (Nomura et al. 2012). However many unanswered questions remain like the design of the linker between the ZFR recombinase and zinc-finger domains may still remain far from optimal.

The greatest achievements in this field have been accomplished with zinc finger nucleases (ZFNs), which comprise an endonuclease domain merged to a zinc finger DNA-binding domain. Current sophisticated methods for creation of altered-specificity zinc finger domains permit ZFNs to be designed to target by a broad range of natural sequences.

Usually ZFRs were prepared by connecting the catalytic domain of a Tn3 resolvase activated mutant to a zinc finger (Zif268) DNA-binding domain. ZFRs can target many new sequences those are being limited by the range of sequences for which specific zinc finger domains (Ramalingam et al. 2013).

FLP-FRT SYSTEM

FLP-FRT is known as one of site specific a recombination

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

system, which is considered as key techniques, play key role in DNA transposition, gene regulation and genetic diversity. The FLP-FRT it is a technique, showing some similarity to cre-lox technology. While the cre-lox system is based on the use of Cre-recombinase, FLP-FRT technology uses flippase (FLP) recombinase, derived from Saccharomyces cerevisiae (Sadowski 1995). Flippase recombinase is an enzyme, which is encoded by 2 μ plasmid.

Flp belongs to ƛ integrase or tyrosine based family of site-specific DNA recombination. Members of this family have got four conserved residues- Arg-191, His-305, Arg-308, Tyr- 343. Similar to Cre-recombinase, Flippase functions are describe as promoting inversions, deletions and insertions of DNA. Flp is known to be one of eukaryotic members of to ƛ integrase family. It is describe, that Flp catalytic domain is similar to Cre catalytic domain only in the 13 % (Kwon et al. 1997). 2 μ circle plasmid is 6318 bp length and has got a two unique regions inside. One of open read frames in 2 μ encodes flippase. Minimum spaces for FLP-FRT are composed of two pairs of repetitions, witch a length of 13bp each, and separated by 8bp length spacer. Base pairs contained within the sequence are a place to binding flippase (Anders et al. 1985, Gronostajewski and Sadowski 1995). After binding to elements of repetitions, FLP could induce bend in the FRT site and than, could promotes exchange of a pair of strands to form a Holliday-like intermediate (Holliday 1964). According Gronostajewskii Sadowski (1985) and Evans et al. (1990) covalent attachment of FLP with a 3'-OH group is in the place, where previously acted phosphotyrosine. Opportunity to join of FLP is made possible by nucleophilic attack of tyrosine 343, upon the scissile phosphodiester bonds of the FRT site. This reaction forms a free 5'-OH group. According to Parsons et al. (1988, 1990), the other amino-acids such as Arg-191, Arg-308 and His-305 are also involved in phosphodiester bond cleavage step.

GENE TARGETING AND APTAMERS

Gene targeting is one of new technologies, which are used to modify endogenous DNA sequence by homologous recombination- important process, inefficient in most organism and cells (Ruff et al. 2014). Aptamer guide technology is new technology, based on exchange of homologous genetic information between a donor molecule and the desired target locus. As reported Ruff et al. (2014), the main limitation of this technique is the low frequency in cells. Aptamers are descirbe as nucleic acid ligands. What is interesting, the name "aptamers" from Latin word aptus, what is mean fit. It is class macromolecules, which has got high affinity and specificity for their target (Levy-Nissenbaum et al. 2008). They are relatively fast and cheap in production process, slow degradation kinetics and- what is important- with low toxicity. Range of aptamers is descirbe between 20 to 80 bp (Levy-Nissenbaum et al. 2008, Wang et al. 1993). They have got high stability under a wide range of buffer conditions, and they are resistant for physical or chemical degradation. What is more, aptamers are susceptible to modifications, especially chemical modifications like radioscopic or fluorescent reporters. Modifications like 2'F or 2'O- Methyl or unnatural L-nucleotides are describe as popular modification of 2'(deoxy)ribose ring modifications. Modification of 3' or 5' groups by thiol groups is known, as methods, which could allow the incorporation of apatamers to nanomaterials. Very important limiting step is low efficiency of homologous recombination, during constructing targeting vectors (Iiizumi et al. 2006). Constructing a vector based appropriate cloning and mapping of genomic DNA fragments. The production of aptamers is selected from a SELEX Technique (Systematic Evolution of Ligands by Exponential Enrichment) (Ellington and Szostak 1990). SELEX was described in early 90's by Ellington and Szostak. The SELEX method used random sequence library (ssDNA or ssRNA which have got length between 20-100 nt). The final randomization gave a diversity of 4n where n corresponding to number of randomized base (Ni et al. 2011). This method went through many improvements, which was created a number of varieties this method, like Toggle-SELEX, Tailored SELEX or Photo-SELEX (Yang et al. 2007). Moreover, the combination of photosensitizer and the aptamer may become an interesting solution used in photodynamic therapy of tumors (Kong and Byun 2013).

Time will tell whether such structures meet the hopes and expectations of researchers and that prove to be effective therapeutic tools (Kong and Byun 2013). Another application of aptamers is, imaging diagnosis of the disease in vivo and in vitro, using an aptamer conjugated to a fluorophore or quantum dot gadolinium, used in magnetic resonance imaging (MRI). The potential diagnostic and therapeutic aptamers fresh impetus, and probably in the near future they will become a standard tool in the hands of clinicians and for biotechnologist's almost unlimited source of ideas and paths to create new businesses and developing existing ones (Esposito et al. 2011).

CRISP

The possibility to make knockout genes became very useful tools, for scientists. One of method for change genes is Crisp-Clustered Regularly Interspaced Short Palindromic Repeats. This system is one of bacteria adaptation form, using short RNA form to direct degradation of foreign nucleic acid. First information about CRISPR was published in early 80's. In 2005 Koonin and colleagues described mechanism of CRISPR step by step

Crisp were described as a repeats, which were found in bacterial and archaeal. Crisp was described as - CRISPR RNAs (crRNAs) which could make complex with Cas-protein (Mali et al. 2013). CRISPR-Cas is one of the most widely-used versions. Cas- protein isolated from Streptococcus pyogenes and sgRNA, described as chimera of two RNA molecules (Jinek et al. 2012, Davis E.2014). Cas9 protein from Streptococcus pyogenes could recognize 3 nt N-G-G sequence, which is describe as PAM (Protospacer Adjacent Motif). PAM is not a par with RNA sequence, but it is located on the same strand, as RNA. DSB could be repair by homologous recombination (HR), but scientist suppose, that non-homologous recombination could repair breaks too.

SUMMARY

The possibility to use a new techniques in molecular biology, gave a new point of view for many evolutionary changes. Possibility to understand how genes are changing and what is happen during mutations could gave chance to understand steps of many diseases.

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

REFERENCE ALBERTS B. 2002. Molecular Biology of the Cell, Fourth Edition. New York: Garland Science | ANDREWS, B. J. , PROTEAU, G. A. , BEATTY, L. G. & SADOWSKI, P. D. 1985 The FLP recombinase of the 2 micron circle DNA of yeast: interaction with its target sequence Cell 40, 795-803. | BERNSTEIN C 1981.Deoxyribonucleic acid repair in bacteriophage. Microbiol. Rev. 45 (1): 72–98. | BERNSTEIN H, BERNSTEIN C, MICHOD R.2011. Meiosis as an Evolutionary Adaptation for DNA Repair. Chapter 19 in DNA Repair. Inna Kruman editor. InTech Open Publisher. | ELLINGTON AD, SZOSTAK JW 1990. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818–822 | ENNIFAR E. , MEYER J. E. , BUCHHOLZ F. , STEWART A. F. SUCK D. 2003. Crystal structure of a wild-type Cre recombinase-loxP synapse reveals a novel spacer conformation suggesting an alternative mechanism for DNA cleavage activation. Nucleic Acid Res: 31(18): 5449-60 | ESPOSITO, C. L. , CATUOGNO, S. , DE FRANCISCIS, V. , CERCHIA, L. , 2011. New insight into clinical development of nucleic acid aptamers. Discov Med. 11(61), 487-96. | EVANS B. R. , CHEN J. W. , PARSONS R. L. , BAUER T. K. , TEPLOW T. B. , GAJ T, MERCER AC, SIRK SJ, SMITH HL, BARBAS CF 3RD. 2013. A comprehensive approach to zinc-finger recombinase customization enables genomic targeting in human cells. Nucleic Acids Res. 41(6):3937-46. | GÓRSKA M. , KOWALSKI M. L. 1997. Kock-out genow- zastosowanie w badaniach medycznych. Alergia. Astma. Immunologia. 1997: 2(3):162-169 | GRONOSTAJEWSKI R. M. , SADOWSKI P. D. 1985. The FLP recombinase of the S. cerevisiae 2 microns plasmid attaches covalently to DNA via a phosphotyrosyl linkage. Mol. Cell. Biol. Vol. 5(11): 3274-3279 | H. Y. KONG AND J. BYUN, 2013. Nucleic Acid Aptamers: New Methods for Selection, Stabilization, and Application in Biomedical Science,. Biomol. Ther. , vol. 21, no. 6, pp. 423–434, | HOESS RH, WIERZBICKI A, ABREMSKI K.1986 The role of the loxP spacer region in P1 site-specific recombination. Nucleic Acids Res.;14:2287–2300 | HOLLIDAY R. 1964. A mechanism for gene conversion in fungi. Genetical research Vol 5(2): 282-304 | IIIZUMI S. , NOMURA Y. , SO S. , UEGAKI K. , AOKI K. , SHIBAHARA K. , ADACHI N. , KOYAMA H. 2006 Simple one-week method to construct gene-targeting vectors: application to production of human knockout cell lines. BioTechniques, Vol. 41, No. 3, pp. 311–316 | KWON H. J. , TIRUMALAI R. , LANDY A. , AND ELLENBERGER, T. 1997. Flexi- bility in DNA recombination: structure of the lambda integrase catalytic cor. Science 276; 126-131 | LEVY-NISSENBAUM E. , RADOVIC-MORENO A. F. , WANG A. Z. , LANGER R. , FAROKHAZAD O. C. 2008. Nanotechnology and aptamers: applications in drug delivery. The Cell. Vol. 26(7):1-8 | LI, X. , & HEYER, W. D.2008. Homologous recombination in DNA repair and DNA damage tolerance. Cell Research18, 99–113 | MALI P., YANG L., ESVELT K., AACH J., GUELL J., GUELL M., DICARLO J., NORVILLE J.E., CHURCH G.M. 2013. RNA Guided human genome engineering via Cas9. Science, 339.823-826 | METZGER, D. FEIL R. 1999.Engineering the mouse genome by site-specific recombinaion Curr. Opin. Biotechnol. 10(5):470-476 | MODESTI, M. , & KANAAR, R. 2011.Homologous recombination: From model organism to human disease. Genome Biology 2, 1014. 1–1014 | NOMURA W, MASUDA A, OHBA K, URABE A, ITO N, RYO A, 2012 Effects of DNA binding of the zinc finger and linkers for domain fusion on the catalytic activity of sequence-specific chimeric recombinases determined by a facile fluorescent system. Biochemistry. 51(7):1510-7. | PARSONS R. L. , EVANS B. R. , ZHENG L. , JAYARAM M. 1990. Functional analysis of Arg-308 mutants of Flp recombinase. Possible role of Arg-308 in coupling substrate binding to catalysis. JBC Vol. 265:4527-4533 | PROROCIC MM, WENLONG D, OLORUNNIJI FJ, AKOPIAN A, SCHLOETEL JG, HANNIGAN A, 2011. Zinc-finger recombinase activities in vitro. Nucleic Acids Res. ; 39(21):9316-28. | RAMALINGAM S, LONDON V, KANDAVELOU K, CEBOTARU L, GUGGINO W, CIVIN C, 2013. Generation and genetic engineering of human induced pluripotent stem cells using designed zinc finger nucleases. Stem Cells Dev. 22(4):595-610. | RUFF P. , KOH K. D. , KESKIN H. , PAI R. B. , STORICI F. 2014. Aptamer-guided gene targeting in yeast and human cells. Nucleic Acid Res. 2014, doi: 10. 1093/nar/gku101 | STARK, W. M. ; BOOCOCK, M. R. 1995.Topological selectivity in site-specific recombinatio". Mobile Genetic Elements. Oxford University Press. pp. 101–29. | STERNBERG N, HAMILTON D. 1981. Bacteriophage P1 site-specific recombination. I. Recombination between loxP sites. J Mol Biol 150:467-86 | WANG K. Y. 2010.A DNA aptamer which binds to and inhibits thrombin exhibits a new structural motif for RNA. Springer | WĘGLEŃSKI PIOTR 2006. Genetyka molekularna. PWN 2009 | WU LC, MAK CH, DEAR N, BOEHM T, FORONI L, RABBITTS TH. 1993.Molecular cloning of a zinc finger protein which binds to the heptamer of the signal sequence for V(D)J recombination. Nucleic Acids Res. 21(22):5067-73. | XIAOHUA NI, CASTANARES M. , MUKHERJEE A. , LUPOLD S. E. 2011. Nucleic acid aptamers: clinical applications and promising new horizons Curr Med Chem. 18(27): 4206–4214 | YANG YYANG D, SCHLUESENER HJ, ZHANG Z 2007. Advances in SELEX and application of aptamers in the central nervous system. Biomol Eng. ;24(6):583-92. Epub | YUENJA W. , YUAN-YEU Y. , PERKINS-BALDING D. , THOMSON J. G. 2011. Recombinase technology: applications and possibilites. Plant Cell rep: 30: 267-285 | ZHENGYAO LEE N.C.,DAFHNIS-CALAS F. , MALLA S.,SMITH M.C. M., BROWN W.R.A.2007.Site-specific recombination in Schizosaccharomyces pombe and systematic assembly of a 400kb transgene array in mammalian cells using the integrase of Streptomyces phage ϕBT1 Nucleic Acids Res.36(1): e9. |