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CRISPR-Cas: Bacterial Immune System (review)

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Abstract
Life threats named Viruses a cellular particles are presents commonly, where archaea and bacteria create the majority of life on Earth too. Accordingly, varied ways of different mechanisms has settled against virus infections. Adaptive immune system afresh discovery known in prokaryotes, which earlier thought to be restricted in higher animals. This system, designated CRISPR-Cas, run sequence-specific adaptive immunity and vitally affect our attentive of host - viruse interaction. CRISPR-depends immunity acting through join in the virus cell's CRISPR locus a short sequences, OK'ing the cell to reminisce, identify and eliminate infections. There has been rapid progression in our thoughtful of this immune system and its uses, but there are numerous properties that await clarification creation the field and inspiring research areas. Highlights of unresolved subjects and overview provides through this review.

INTRODUCTION

One cell encountered 10 viruses on Earth [1]. Viruses a key factor by genetic exchange which performing originators and predators in the ecology and evolution of life. Not astonishing, both arms of immune system (innate and adaptive) countermeasures while its perceived in hosts. Systems that Innate (non-specific) distinguish definite genetic structures of foreign organisms. Prokaryotes immune steps include Restriction alteration and abortive infection, whereas adaptive systems, on the other side, pathogens specific features could be diagnosed by this system. B and T lymphocytes could impart to realize cells components with a view viruses and stomachic cells. Because of the intricacy of immune components, evaluation of prokaryotes adaptive arm [2] was amaze. Region located in prokaryotic DNA named (CRISPR) Clustered Regularly Interspaced Short Palindromic Repeats [3]. In a cells this system functioned that chiefly stand-alone, an inevitability in those singular cell microorganism. RNA or DNA targeted by CRISPR-Cas system become a method of defense versus many pathogens [2,4]. CRISPR database revealed that CRISPR locus detected in *E. coli* firstly [5], and existing in 84% and 45% for both is archaea and bacteria respectively [6]. Investigation explained samples prejudice effects on occurrence variation of bacteria 20 times than archaea. Sequence of shortened repeats existed in CRISPR disconnect via spaces with sequence exceptions. Plasmids and viruses nucleic acids yields spacers, which establish to the augment that anti-viruse system give rise CRISPR [7]. In every spacers addition the new viruse could be eliminated. Neutralizing and destroying all viruses because the spacers behave as factor of recognition.

Genes of CRISPR associated with (cas) presents close to CRISPR that important for some immune proteins codding and affected by CRISPR action [2, 10]. Progeny gets via inheritance the safety due to generic alteration of spacer processing. Spacers newly added in one CRISPR ends, record chronologically created in viruses also happens in new born genomes.

Three main steps arbitrated the CRISPR-Cas process (Figure 1). First step, adaptation, CRISPR locus gained new spacers via insertion of target (Figure 2). Second, expression, cas genes expression then CRISPR transcription generated long precursor CRISPR RNA (pre-crRNA). Auxiliary factors and cas proteins associated in the pre-crRNA maturation to crRNA. In last stage, interference, the two factors crRNA and Cas proteins affecting the recognized nucleic acid is and finally destroyed.

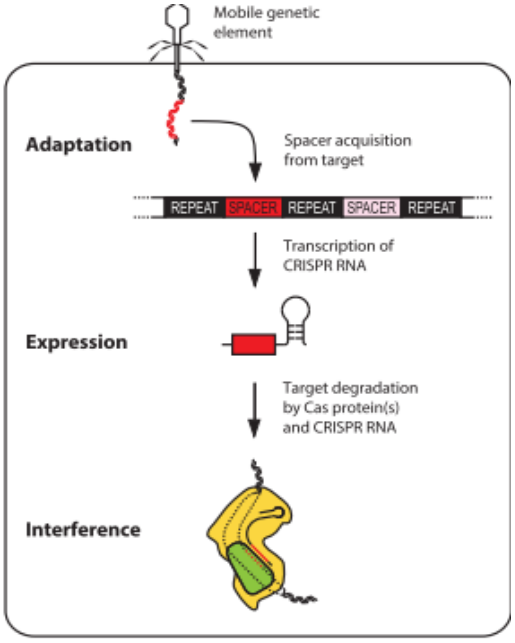

Fig: 1

Fig. 1. CRISPR-Cas immunity key stages. 1) Adaptation: CRISPR locus gets a new spacers. 2) Expression: CRISPR locus transcription also CRISPR RNA processing. 3) Interference: Cas protein(s) and CRISPR RNA detected and degraded of mobile genetic elements.

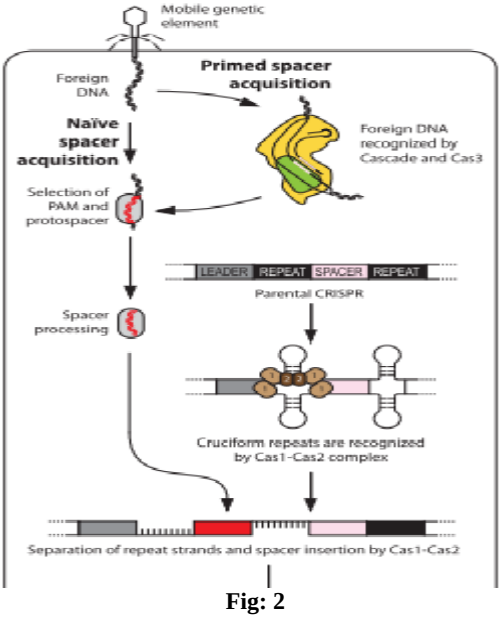

Fig: 2

Fig. 2. I-E system adaptation typically. Spacer acquisition located in 2 types, naïve and primed. PAM (Protospacer Adjacent Motif) must be existed in the two types and on Cas1-Cas2 complex dependence completely. CRISPR recognition done by the Cas1-Cas2 complex and possible formulates it for spacer integration. If

no earlier data concerned the target in the CRISPR acquisition of Naïve spacers will happens. The CRISPR locus needed for acquisition of Primed spacer in that fits the target DNA also Cascade complex and Cas3 incidence. Ant insertion of genetic mobile element in spacer occurred in Primed acquisition.

We runs in this review CRISPR immunity mechanism and uniformity, ecologic and evolutionary of viral infection. Covering of function that naturally occurred based on immunity and interesting fresh progression of CRISPR-Cas related to genetically vibes and their enforcement.

1. CRISPR-Cas System Variety, Evolutionary and Ecologic views

Repeats lengths and sequence also the spacer's lengths are fine preserved in a CRISPR loci, but in the same or diverse genomes could diverge between CRISPRs. Sequence repeat and spacers are in lengths 21 to 48 base pair and 26 to 72 base pair respectively [3,6,7]. Variety that witnessed in the system explain the extensiveness. The total of spacers within a CRISPR locus differ broadly; from a little to quite a lot of hundred [6]. CRISPR loci can be singular or many in Genomes as well as that loci of other species can constitute a substantial chromosomal section. In *Methanocaldococcus* sp. FS406-22 and *S. tokodaii* strains had seven genomics with 18 CRISPRs and 191spacers and 5 CRISPRs and 458 spacers respectively, which referred to 1% genomic CRISPRs composition [11]. Cas genes doesn't existed neighboring to CRISPR loci in all times and in its place trust on transen-coded elements. Leader sequences was noticed in many species such as *M. jannaschii*, *A. fulgidus* and *M. thermautotrophicus*. Several Cas proteins are recognized to nucleic acids cooperation, furthermore, its elaborate and essential in adaptation [3]. Several cas proteins are recognized to nucleic acids cooperation, furthermore, its elaborated and essential in adaptation. [12].

2. CRISPR adaptation. .

Genomic memory offers via the adaptation phase it's a precondition for the next two phases (expression and interference) so counterbalance nucleic acids attacking with respect. Demonstration of new spacers insertion in many CRISPRCas subtypes; Type I-A (*S. solf*-'[p;08uataricus [32], and *S. islandicus* [33]), I-B (*H. hispanica* [34]), I-E (*E. coli* [35]) , I-F (*Ps. aeruginosa* [38] , *Pecto. atrosepticum* [39]) ,Type II-A (*S. thermophilus* [2] and a *Str. pyogenes* system expressed in *Staph. aureus* [20]). Spacer acquisition located in 2 types; naïve, means the virus has not been beforehand faced, and primed, refers to t pre-existing invader recording of the CRISPR (Figure 2) [40].

Mechanism of spacers acquisition partially understood for all that it was observed. Theoretically, there are two steps of this process: selection of protospacer and spacer material generation then creation of new one throughout spacer and CRISPR integration. CRISPR size limitation is necessary via spacer's deletion, but a slight information's of this pathway regularity of that actions. Cas1 and Cas2 are the spacer integration key factors. These proteins were abundant but their functions unessential for abrasion [10]. Performance of confirmation showed latter via *E. coli* I-E system cas proteins overexpressed, even in such cas proteins deprivation integrates of spacers was resulted [36]. In above bacteria cas proteins were nucleases [40] and any integration canceling of spacers could happens via Cas1active site mutation [36,37]. Cas1 and Cas2 procedure *Escherichia coli* considered a complex connection where dimers formed from 1 Cas2 connects 2 Cas1 dimers. Nuclease action of Cas2 could be unnecessary while spacer integration needs for form complex figuration. In entrusted pattern CRISPR DNA connected in Cas2 by Cas1 tendencies, additional assisted play a candid part in acquisition of spacer [39]. It's might that spacer integration meditated by both complexes Cas1 & Cas2 throughout spacer material transporting, so that clarify the needing in complex formation many subunits of Cas1. Spacer acquisition also required supplementary factors: in Type II-A system Cas9, Csn2 also tracrRNA [2 ,20] and Type I-B Cas4 [34]. Integration mechanization perhaps leads via Cas9 but Csn2, racrRNA and Cas4 roles were unclear.

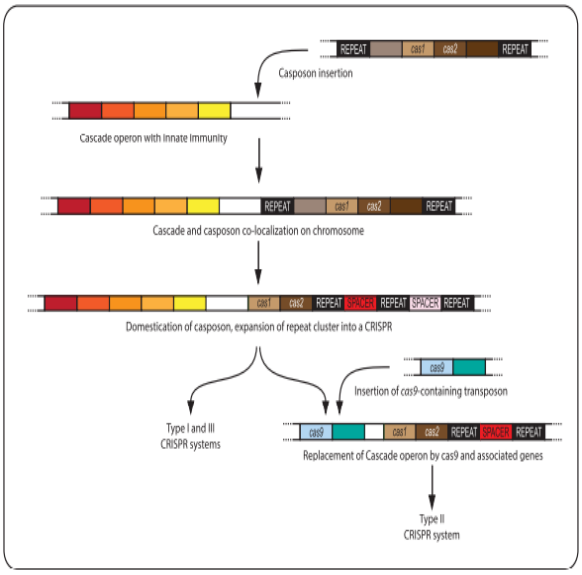

Fig 3

Fig. 3. Hypothesis the evolution of CRISPR-Cas system. Cascade operon become neighboring to casposon via insertion capability targeting of innate immune proteins. Both Types (I and III) of CRISPR_Cas systems formed by expanding the terminal inverted repeats (TIRs) cluster of CRISPR and that subsequent of casposon loose genes. Replacing of Cascade genes to transposon cas9 become developmental from origination of Type II. Nature variety generate unsigned genetically elements.

Interference machinery joined with adaptation via primed spacer acquisition, if CRISPR contents have targeted spacer the interference joining occurs. Acquisition could be quicken by pre-existing spacer and mechanization of interference of the next target spacer. Designation of the Primed spacer acquisition firstly occurred in system I-E *E. coli* [37], but *H. hispanica* I-B also has reported [34] further *P. atrosepticum* I-F [39], whereas Type II or III system included but not so far. Priming appears to arise by somewhat dissimilar actions in the termed situations identifying of these mechanisms still unknown. Cas2 in I-F Type, can fuse with Cas3 [13], additional designating happens in adaptation when directly connects with interference. In attention, mismatching occurs in spacers so that spacers' protection to acquisition of primed spacer gets poorly supply. We could saw that in system II-A spacer acquisition needs Cas9, spacer acquisition not considered an example here because condition doesn't strict in case target versus spacer (pre-existing) [20].

3. Genes Expression of CRISPR RNA and cas. .

Immune microorganism CRISPR-Cas loci Transcripting generate RNA protein guide and have variances in many certain types. Transcribing of the CRISPR locus in entirely systems; (crRNP) CRISPR_ribonucleoprotein results from ribose nucleic acid processed by Cas ribonucleases. CRISPR Transcripting in certain bacterial species, (*E. coli*, *Py. furiosus* and *Sulfolobus* sp.), creates in specific main regions [26].

Promoter elements in the leader and controlling proteins of binding locations, as well as elements vital in integration of spacer. Generation of pre-crRNA that primarily transcripting, also it's includes hairpins as ordinary structures in series if CRISPR contains palindromical repeats. Processing of the pre_crRNA to

minor parts equivalent to a solitary spacers bordered via limited repeats. Processing is made via the Cas protein, and differs with the subtype, if that protein is part of a complex or not. Pre-crRNA of owns CRISPR they don't processing themselves until they are existed in three types [38].

The 3 kinds systems of CRISPR-Cas in overall evaluation displays that resemblances in pre-crRNA processing of Type I and III systems sharing in addition to in the crRNP complexes formation of structures (Fig. 3). Utilizing a Cas6 protein in all Type I and III systems for processing of pre-crRNA excluding Type I-C, that hires Cas5d [39]. System presents in I-E *E. coli*, the pre-crRNA is sliced in a mentalist sovereign pathway via the Cas6e endoribonuclease [10].

E. coli Cascade complex employs by the key components of Cas6e and the crRNA, which moreover holds 1 version of Cse1, 2 versions of Cse2, 1 version of Cas5e and 6 versions of Cas7 [37]. Sea-horse figure sees in Cascade where a helical provided along backbone of Cas7 proteins which the crRNA was showed [40] site via hairpin thumbs prolonging from Cas7. Anchoring the 50 and 30 ends of the Cas5e and Cas6e crRNA to reverse edges of Cascade. A research confirmed that Cascade might gathered in cells missing crRNA and then in vitro was filled with crRNA.

D. Rath *et al*. and Biochimie suggested the fact gathering of crRNA processing and Cascade don't need to be an integrated process. Systems of Type III, steps for processing of specific cr_RNA. Sequence concerned with pre- maturation could arbitrated via Cas6, then trimmed sequence (ruler-based) of unspecific crRNA to harvesting crRNAs in the 30 end which matured with known 50 end and mutable 30 end. III-A (Csm) and Type III-B (Cmr) complexes disclose types backbone showed in structural analysis, somewhat similar to Cascade, in other hand crRNA be aligned. Csm1-Csm4/Cmr2-Cmr3 and Csm5/Cmr1-Cmr6 of 30 end anchored via the End 50 of the crRNA [23,25]. Whole different pathway serviceable by system II thus RNase III of the host along with little RNA (tra-crRNA) sharing the processing for biogenesis of crRNA, which pairing with [16] (Fig.3). Furthermore, Cas9 protein important for processing system II [16, 18], so their precise action indistinct. crRNA have obvious 50 trimming of II systems via undefined nuclease when Cas9 stills attached to crRNA-tra-crRNA [18].

4. Interference

CRISPR-Cas systems targets interference principle by binding Cas protein to crRNA which triggering the targeted degradation when protospacer corresponding localized (Fig. 3). Certain nucleases of Cas helps in degradation [10, 17].Targeted DNA locates Cascade in Type I systems but the interference happens by Cas3 nuclease/helicase [10, 13]. Binding of target against Cascade revealed recruiting of Cas3, or become a perpetual section in instance of Cascade Type I-A. Distinct genes encoding domains of helicase and Cas3 of Type I-A [13]. Genes in case I-B systems splitting cas3 according to Bioinformatics data [13], but another data in experimental work designated that there is no permanent action. Though, the targeted DNA (double stranded) in Type I damaged collectively through the two domains [34].

Seeds regions in the systems Type I and II interference contains PAM sequences and complementarity of crRNA protospacer faultless, which situated PAM to nearby. Self CRISPR loci offensive inhibition by PAM triggers "non-self-activation. Positioned out sixth base Cas7 thumbs bearing crRNA in the Cascade which subsequently incongruities doesn't cause targets binds in the places [40]. Interference not effecting with a little more disparity exterior seed region. Non-specific way interaction of DNA-Cascade can occurred [30] which searching for seed regions and PAMs, with Cse1 advising to be checked via PAM. The lefts parts of crRNA pairing is next to Seed-region bp, resulting to shifts the free strands of DNA to creates R-loop shape. By the way imperative role plays by Cse1. True relations in both targets DNA and Cascade from binding of CrRNA to the target [22] consequent to triggering enrolment Cas3. Later Cas3 incisions the DNA targeted and incomes with liberal dilapidation DNA whilst Cascade seemingly distances also is prepared for acting again.

Cas9 protein is necessary in Type II systems to interfere, but its desires crRNA, Cas9 binds to a tracrRNA and crRNA for performing targets recognized and degrading, this system type dissimilar for the two types (I, II) [16]. Species of *S. pyogenes* and *A. naeslundii*, their Cas9 disclose lobe to recognizing and nuclease action, during interface crRNA-DNA heteroduplex accommodating in a charged positively appeared of groove. Connecting between DNA and crRNA crucial occurs via lobe of recognition, HNH and Ruv C nucleases proteins presents in nuclease lobe which slice target strands that complementary and none, congruently. Structure lobes recognizing induction of crRNA, so DNA substrates become able to binding, advising of Cas9 activation is processed via crRNA loads [20].

Six dissimilar proteins presents in the Csm complex in Type III-A but the recognition of nuclease is not done yet [23]. Six or seven various proteins existed in the Cmr complex in III-B [23, 24] and the target the cleaved by Cmr4 protein. Primary data showed Csm and Cmr complexes that targets DNA plus RNA respectively [4,25], also the full understood still under investigation.

Csm complex targeted the RNA in species *Thermus thermophilus* and *S. thermophilus*, while both types III-A and III-B presents in *Thermus thermophilus*, sharing of crRNA through the complexes Csm and Cmr. *S. islandicus* has similar Cmr complex which utilized as DNA Targeting. Type III revealed no PAMs, instate the self and non-recognition is attained via crRNA prostration bp in to steward DNA repeat, in case resulting "self-inactivation", un operation that basically dissimilar to Type I and II PAM discrimination.

Staph. aureus has unique feature in interference, wherever the types is banned of offensive targets to un-transcripting e.g. lysogenized phages. That system brands action because the effective degrading will stops host's chromosome. The mechanism behind the system is not known and it leftovers could be resolute when be the backbone of CRISPR-Cas systems [40, 37].

5. Anti-CRISPR mechanisms.

Strategies developed by cells to counter viruses, countermeasures established via the viruses to these strategies. CRISPRCas counteracting mechanisms in several manners for that system have been designated. Random mutagenesis is the greatest basic method in avoidance the powerful action CRISPR-Cas in these viruses which employs via affecting the main steps of interaction and recognizing [40]. Other advanced in Type I-E and I-F systems has been noticed that encoding phages of *P. aeruginosa* affecting the activity of some proteins in that system. These proteins functions not obviously appeared to be effective against Cas-proteins and crRNA expression. Probable, this proteins interrupts the connection between CRISPR-Cas complexes [37]. In other words, studies referred that system could be utilized by the phages to excessing the infection. Phage of *Vibrio cholerae* (ICP1) hold system CRISPReCas from I-F Type which targeting loci in hosts; in PLE there is anti-phage mechanism [35].

After phages genome get inside of the cell, cas genes and viral crRNAs expression occurred to make the infection succeeded inside the host. When the engineering occurs inside a host or specifically in CRISPR, that means the virus system shorter matches the PLE, no sense to ICP1 infection in the next time. Little bacteriophages achieve to cause disease via catching up new spacer to targets locus of the host, which indicates ability of viruses to usage potentially adaptive CRISPRCas system.

6. CRISPR applications.

CRISPR is render to an auspicious promising actor in medically concerned genetic items due to its versatility, reliability and specificity. Screening of CRISPR could be practicable in many studies genetic function associated like genome identification that important for cell subsistence, resistance for medications and extracellular compounds as well cancer metastasis. Designing of sgRNA/Cas9 to upcoming enhancements structure, picking also transmission would aided in progression particularly and efficacies of secerns. CRISPR screen powerful implementations in health projects contain spaces like transcriptome screen, a live imagined genomics associated with screening of CRISPR, Cas9 inducement which aids certain timing screening. Helps in improvement for whole subject that would expectantly envision the next time CRISPR screen maybe renovate functional genetic works.

Dairy cheese production, there is Danisco as enhancer factor as culturing market. Certain bacterial species that generically holds a CRISPRs that resistant to phages which harmful to products. As immunity mechanism the company use it to give further important of CRISPR-Cas [2]. Though, the influential properties of Type II speed the development of eukaryotes. Chiefly gathering of the two type's crRNA and tra-crRNA yields (sgRNA) single guide RNA cemented the pathway of expansion [18]. Rather than Cas3, which cause damaging to the targets, while Cas9 gives a single double-stranded break of the DNA with further useful tools in and genetic editing. Gene modifications appeared to be useful in DNA fixing of eukaryotes.

Cas9 potential therapeutic has been verified. Cas9 as antimicrobial agent that has been developed to eradicate and stop the different mechanism of virulence bacterial species. Human illness like cystic fibrosis had applied in genetic therapy apps through repairs of cftr gene in cultured media, germ line alteration DNA in mouse - cells leads to curative action dominant cascade disorder and dystrophy of Duchenne muscular, while in adult mice by curing hereditary tyrosinemia [30]. Furthermore, Cas9 could healing sever viral infection like hepatitis and human immune-sorbent viruses. A known version of Cas9 could be verified in order to delivering via Adeno-associated virus, another somatic therapeutical purpose based on gene alteration becomes critical and genetic excision which accurate embryos improvement. [33]. So that keep close to human curing when animal model utilized which reduce moral arguments. Gene regulation Programming utilized systems of CRISPR-Cas. Gene silencing through interfering elongation or RNA polymerase binding becomes true with utilizing mutation of Cas9 (dCas9) and efficient of Cascade and a nuclease [40].

CONCLUSIONS

The development research of CRISPR field has increased rapidly. Much gaining of knowledge, and the field is magnetize a cumulative amount of consideration. Though, partial knowledge we provides for several key questions, mainly with admiration of integrated to spacer mechanisms. Adaptation in primed spacer acquisition and interference connection becomes additional unanswered requests. The frequency CRISPR horizontal transfer reasons also remnants would be studied massively, just like in evolutionary and regulation in CRISPR-Cas model. Additional projects merit in ecology factors of CRISPR, CRISPR-Cas role in processes that non-immune dependent. Different laboratories give attention to the Cas9 monotonous, with more acceptance importance. Recent superior apps in advanced, as well as the shining future will comes with medical benefits of CRISPRCas systems.

REFERENCES

[1] C.A. Suttle. Viruses in the sea, Nature 437 (7057) (2005) 356e361.

[2] R. Barrangou , Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P.CRISPR provides acquired resistance against viruses in prokaryotes, Science 315 (5819) (2007) 1709e1712.

[3] R. Jansen , van Embden JD, Gaastra W, Schouls LM..Identification of a novel family of sequence repeats among prokaryotes, OMICS 6 (1) (2002) 23e33.

[4] R. Caryn Hale, Peng Zhao, Sara Olson, Michael O. Duff, Brenton R. Graveley, Lance Wells, Rebecca M. Terns and Michael P. Terns.RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex, Cell 139 (5) (2009) 945e956.

[5] Y Ishino, H Shinagawa, K Makino, M Amemura, and A Nakata. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product, J. Bacteriol. 169 (12) (1987) 5429e5433.

[6] I. Grissa, G. Vergnaud, C. Pourcel, CRISPR Finder. A web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res.35 (Web Server issue) (2007)W52eW57. .

[7] D. Henrik, Møller, Lance Parsons, Tue S. Jørgensen, David Botstein and Birgitte Regenberg. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin, Microbiology 151 (Pt 8)(2005)2551e2561.

[8] SJ. Brouns , Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes, Science 321 (5891) (2008) 960e964.

[9] Rousseau Christine , Mathieu Gonnet, Marc Le Romancer and Jacques Nicolas. CRISPI: a CRISPR interactive database, Bioinformatics 25 (24) (2009) 3317e3318.

[10] Makarova Kira S. , L. Aravind, Nick V. Grishin, Igor B. Rogozin and Eugene V. Koonin .A DNA repair system specific for thermophilic Archaea and bacteria predicted by genomic context analysis, Nucleic Acids Res. 30 (2)(2002)482e496.

[11] KS. Makarova, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF, van der Oost J, Koonin EV. Evolution and classification of the CRISPR-Cas systems, Nat.Rev.Microbiol.9(6)(2011)467e477.

[12] E.V. Koonin, M. Krupovic. Evolution of adaptive immunity from transposable elements combined with innate immune systems, Nat. Rev. Genet. 16 (2014) 184e192.

[13] T Sinkunas , Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys V. Cas3 is a single-stranded DNA nuclease and ATP dependent helicase in the CRISPR/Cas immune system, EMBO J. 30 (7) (2011)1335e1342.

[14] Deltcheva Elitza , Krzysztof Chylinski, Cynthia M. Sharma, Karine Gonzales, Yanjie Chao, Zaid A. Pirzada, Maria R. Eckert, Jörg Vogel and Emmanuelle Charpentier. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III, Nature 471 (7340) (2011) 602e607.

[15] JE. Garneau , Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadán AH, Moineau S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA, Nature 468 (7320) (2010) 67e71.

[16] M. Jinek, Chylinski K., Fonfara I, Hauer M., Doudna J.A., Charpenier J. A .A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science 337 (6096) (2012) 816e821. .

[17] E.V. Koonin, K.S. Makarova. CRISPR-Cas: evolution of an RNA-based adaptive immunity system in prokaryotes, RNA Biol. 10 (5) (2013) 679e686.

[18] K. Chylinski, A. Le Rhun, E. Charpentier. The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems, RNA Biol. 10 (5) (2013) 726e737.

[19] C. Rouillon , Zhou M, Zhang J, Politis A, Beilsten-Edmands V, Cannone G, Graham S, Robinson CV, Spagnolo L, White MF. Structure of the CRISPR interference complex CSM reveals key similarities with cascade, Mol. Cell. 52 (1) (2013) 124e134.

[20] M. Spilman , Cocozaki A, Hale C, Shao Y, Ramia N, Terns R, Terns M, Li H, Stagg S.Structure of an RNA silencing complex of the CRISPR-Cas immune system, Mol. Cell. 52 (1) (2013) 146e152.

[21] H.J. Raymond. Staals, Yoshihiro Agari, Saori Maki-Yonekura, Yifan Zhu, David W. Taylor, Esther van Duijn, Arjan Barendregt, Marnix Vlot, Jasper J. Koehorst, Keiko Sakamoto, Akiko Masuda, Naoshi Dohmae, Peter J. Schaap, Jennifer A. Doudna, Albert J.R. Heck, Koji Yonekura, John van der Oost, and Akeo Shinkai. Structure and activity of the RNA-targeting Type III-BCRISPR-Cas complex of Thermus thermophilus, Mol. Cell. 52 (1) (2013)135e145.

[22] U Pul , Wurm R, Arslan Z, Geissen R, Hofmann N, Wagner R.. Identification and characterization of *E. coli* CRISPR-cas promoters and their silencing by H-NS, Mol. Microbiol. 75 (6) (2010) 1495e1512.

[23] G.W. Tyson, J.F. Banfield. Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses, Environ. Microbiol. 10 (1) (2008) 200e207.

[24] A.F. Andersson, J.F. Banfield. Virus population dynamics and acquired virus resistance in natural microbial communities, Science 320 (5879) (2008)1047e1050.

[25] Paez-Espino D, Morovic W, Sun CL, Thomas BC, Ueda K, Stahl B, Barrangou R, Banfield JF..Strong bias in the bacterial CRISPR elements that confer immunity to phage, Nat. Commun. 4 (2013) 1430.

[26] R. Levin Bruce , Sylvain Moineau, Mary Bushman and Rodolphe Barrangou .The population and evolutionary dynamics of phage and bacteria with CRISPR-mediated immunity, PLoS Genet. 9 (3) (2013) e1003312.

[27] JK. Nuñez , Lee AS, Engelman A, Doudna JA. Integrase-mediated spacer acquisition during CRISPR-Cas adaptive immunity, Nature(2015).

[28] S. Erdmann, R.A. Garrett, Selective and hyperactive uptake of foreign DNA by adaptive immune systems of an archaeon via two distinct mechanisms, Mol. Microbiol.85(6)(2012)1044e1056.

[29] T Liu , Li Y, Wang X, Ye Q, Li H, Liang Y, She Q, Peng N. Transcriptional regulator-mediated activation of adaptation genes triggers CRISPR de novo spacer acquisition, Nucleic Acids Res. (2015).

[30] M Li , Wang R, Zhao D, Xiang H. Adaptation of the Haloarcula hispanica CRISPR-Cas system to a purified virus strictly requires a priming process, Nucleic Acids Res. 42 (4) (2014)2483e2492.

[31] DC. Swarts , Mosterd C, van Passel MW, Brouns SJ. CRISPR interference directs strand specific spacer acquisition, PLoS One7(4)(2012)e35888.

[32] KC Cady , Bondy-Denomy J, Heussler GE, Davidson AR, O'Toole GA. The CRISPR/Cas adaptive immune system of Pseudomonas aeruginosa mediates resistance to naturally occurring and engineered phages, J. Bacteriol. 194 (21) (2012) 5728e5738. [33] C Richter , Dy RL, McKenzie RE, Watson BN, Taylor C, Chang JT, McNeil MB, Staals RH, Fineran PC. Priming in the Type I-F CRISPR-Cas system triggers strandindependent spacer acquisition, bi-directionally from the primed protospacer, Nucleic Acids Res. 42 (13) (2014) 8516e8526.

[34] P.C. Fineran, E. Charpentier, Memory of viral infections by CRISPR-Cas adaptive immune systems: acquisition of new information, Virology 434 (2)(2012)202e209.

[35] M Babu , Beloglazova N, Flick R, Graham C, Skarina T, Nocek B, Gagarinova A, Pogoutse O, Brown G, Binkowski A, Phanse S, Joachimiak A, Koonin EV, Savchenko A, Emili A, Greenblatt J, Edwards AM, Yakunin AF. A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair, Mol. Microbiol. 79 (2) (2011) 484e502.

[36] JK Nuñez , Kranzusch PJ, Noeske J, Wright AV, Davies CW, Doudna JA. Cas1-Cas2 complex formation mediates spacer acquisition during CRISPR-Cas adaptive immunity, Nat. Struct. Mol. Biol. 21 (6) (2014) 528e534.

[37] FJ Mojica , Díez-Villaseñor C, García-Martínez J, Almendros C. Short motif sequences determine the targets of the prokaryotic CRISPR defence system, Microbiology 155 (Pt 3) (2009) 733e740.

[38] MÈ Dupuis , Villion M, Magadán AH, Moineau S. CRISPR-Cas and restriction-modification systems are compatible and increase phage resistance, Nat. Commun. 4 (2013) 2087.

[39] Z. Arslan , Hermanns V, Wurm R, Wagner R, Pul Ü. Detection and characterization of spacer integration intermediates in type I-E CRISPR-Cas system, Nucleic Acids Res. 42 (12) (2014) 7884e7893. [40] C. Peter. Fineran, Matthias J. H. Gerritzen, María Suárez-Diez, Tim Künne, Jos Boekhorst, Sacha A. F. T. van Hijum, Raymond H. J. Staals and Stan J. J. Brouns. Degenerate target sites mediate rapid primed CRISPR adaptation, Proc. Natl. Acad. Sci. U. S. A. 111 (16) (2014) E1629eE1638.