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Genome Editing in Space with Emphasis on the Role of CRISPR Cas9

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ABSTRACT

Astro-microbiology is the study of microorganisms in outer space. Considered to be an interdisciplinary subject, Astro-microbiology has its foundational information in subjects like Astro-biology as well as microbiology. As microorganisms are exposed to several galactic and electromagnetic radiations in outer space, they are bound to undergo mutations. These mutant microorganisms influence the space explorers as well as might be carried back to Earth. The study of Astro-microbiology finds its utmost significance in protecting the health of space explorers and human health on this planet as well. The genetic material of astronauts is prone to damage on exposure to the harmful galactic radiation. The CRISPR Cas9 systems have enabled researchers to develop a CRISPR based assay to study these DNA damages and initiate repair of the target genes. The main aim of our review is to compile different facts associated with DNA damage and its consequent repair. Employing these techniques in outer space and the future of gene editing are also core components of our review.

Keywords: Astro-microbiology, DNA damage repair, CRISPR Cas9, NHEJ, HDR, MMEJ, SSA

INTRODUCTION

With rapid advancement of space science and technology, the diverse field of space microbiology is one the most significant concerns of space scientists. The microbes found in the space environment are integrally related to human activity. As microorganisms are a major part of our life on Earth, during subsequent space travel there are a significant number of microbes that get transported to outer space. These microorganisms are very efficient in terms of adaptability. They adjust readily to the changing environmental conditions in outer space however this adaptability comes with pros and cons. Some changes might be benefitting while others may potentially become a threat. Still in the most nascent stages, space microbiology is a thriving research domain that is being explored gradually. Several factors associated with the conditions in the space flight is a major problem and a barrier in terms of conducting significant experimental research however in order to overcome such limitations, space like conditions have been simulated on ground by researchers using cutting edge technologies that enable them to study the behavioural pattern of space microbes in real space time conditions [1].

The NASA Science plan's major component: the microbiology wing plans to study the effect of spaceflight on microbial life, metabolic and associated processes. The various factors to be studied involved the influence of the space environment on microbial interactions, metabolic pathways and development of biofilms. There are quite a number of experiments initiated in order to track the degree of persistence of microorganisms in space crafts, biofilm formation, pathogenicity and virulence. However, it is very difficult to mimic the space flight conditions like the development of microgravity is both time consuming and requires huge amounts of both resources, finances and cutting-edge technologies as well [2].

FACTORS RESPONSIBLE FOR DNA DAMAGE IN ASTRONAUTS

As the space explorers travel and investigate past the defensive line of Earth, there is a high gamble of DNA harm because of the openness to monstrous ionizing radiation (IR) and modified gravitational power. Single strand breaks (SSBs) and double strand breaks (DSBs) emerge when IR influences DNA [3]. It has been seen in space travellers that a few changes were brief and some might maintain. Radiation as well as weightlessness, rest timing, prohibitive eating regimen affect DNA as well as the entire body. DNA harm might be expanded upon longer openness in the spaceflight [4].

CRISPR CAS9 IN VITRO

Discovered in the beginning of this decade CRISPR Cas9 is one of the most promising tools for repairing and editing damaged DNA. The CRISPR is a specific region of the bacterial genome that contains a gene cluster of several interspaced regular palindromic repeats. In addition to the CRISPR loci, adaptive immunity is also dependent upon a set of CRISPR associated genes, known as Cas genes. Abbreviated as "clusters of regularly interspaced short palindromic repeats", CRISPR was found in *Escherichia coli* for the first time. CRISPR Cas9 comprises short segments of palindromic DNA, nucleotide repeats and spacers as well. These spacers have a special function that serves as a memory to the bacteria. Spacers help to store information about a past viral attack and warn the bacteria in advance on encountering a similar attack in the future. A part of the viral genome attacking the bacteria is inserted within these spacers. The integration of the viral genome in the spacer of CRISPR is further translated to produce the CRISPR RNA and these CRISPR RNAs become the guide RNA that helps to detect problematic or erroneous sequences in the host genetic material [5]. A wide range of CRISPR Cas9 proteins have been discovered till date and a comparative analysis of these proteins are presented in **Table 01**:

Table 01: Comparative analysis of some popular CRISPR Associated Proteins or Cas Proteins

Sl. No.	CasProtein	Features	Reference
01.	**Cas 3**	It utilizes ATP for reducing DNA into shorter nucleotides thus consequently removing the invader DNA [38].	He L, St John James M, Radovcic M, Ivancic-Bace I, Bolt EL. Cas3 Protein-A Review of a Multi-Tasking Machine. Genes (Basel). 2020, Feb 18;11(2):208. PMID: 32085454; PMCID: PMC7074321. doi:10.3390/genes11020208.
02.	**Cas 9**	Coupled with a guide RNA (gRNA), both form a binary complex that targets DNA at specific sites (requiring repair) and cleaves both the strands, followed by repair by cell's own inbuilt mechanisms [7].	Fuguo Jiang, Jennifer A. Doudna, "CRISPR - Cas9 Structures and Mechanisms": Annual Review of Biophysics, Vol. 46:505-529 publication date May 2017. doi: 10.1146/annurev-biophys-062215-010822.
03.	**Cas 12**	It targets dsDNA, produces staggard cuts. It is often activated by a target DNA molecule that matches its spacer sequence after the chopping of ssDNA [39].	Leung RK, Cheng QX, Wu ZL, Khan G, Liu Y, Xia HY, Wang J. CRISPR-Cas12-based nucleic acids detection systems. Methods. 2021, Mar 2:S1046-2023(21)00063-3.PMID: 33662563. doi:10.1016/j.ymeth.2021.02.018.
04	**Cas 13**	It is activated by ssRNA and once activated it unveils RNAse activity and destroys other allied RNAs regardless of the sequence. It has wide range of therapeutic application [40].	Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdine V, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F. RNA targeting with CRISPR-Cas13. Nature. 2017 Oct 12;550(7675):280-284. EpubOct 4, 2017. PMID: 28976959; PMCID: PMC5706658. doi:10.1038/nature24049.
05.	**Cas 14**	It uses similar mechanism as that of Cas 12 and Cas 13, but Cas 14 is highly specific. It binds to ssDNA and requires a gRNA but unlike Cas9 there is no need for PAM [41].	Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, Cofsky JC, Kyrpides NC, Banfield JF, Doudna JA. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science. 2018 Nov 16;362(6416):839-842. Epub 2018, Oct 18. PMID: 30337455; PMCID: PMC6659742. doi:10.1126/science.aav4294.

CRISPR CAS9 SYSTEM AND ITS ROLE IN DNA REPAIR

Target specific genome editing involves introduction of sequence specific breaks and use of CRISPR Cas9 systems is considered to be the best approach [6]. During CRISPR/Cas9 mediated genome editing (Figure 1), the Cas9 nuclease is guided by a guide RNA or gRNA to perceive and make a double-stranded break at a particular site in the genome. The CRISPR Cas9 systems contain a single guide RNA (sgRNA) which is composed of CRISPR RNA and small trans encoded crRNA and a double strand nuclease. crRNA which is 20bp length has a sequence complementary to the target region of a genome and usually has non homologous sequence for sequence specific editing. The trans encoded RNA forms a complex with CRISPR RNA by hybridization.

This trans encoded CRISPR RNA complex on binding with the Cas9 protein is activated and is now considered as the DNA binding structure. A Protospacer Adjacent Motif (PAM) which is 3-5 nucleotides downstream from the target sequence is required for binding of Cas9 and consequent cleavage of DNA resulting in blunt ends [7]. The off-target events associated to CRISPR Cas9 can be reduced in multiple ways [8]. To sort out this problem, a few Cas9 endonucleases have been engineered to have a (Non-Homologous End Joining) repair pathway (***Figure 2***) [9] or the HDR (Homology Directed Repair) pathway (***Figure 3***) [10]. Non-homologous end joining may introduce arbitrary insertions or deletions at the break site, on the other hand, homologous recombination can be utilized to make particular changes to the DNA sequence through an engineered repair template. Though NHEJ repair pathway shows lower levels of fidelity as well as is more error prone but has more prominence over the HDR pathway. Less sequence homology and the non-requirement of template RNA makes NHEJ a faster mode of repair and the repair occurs throughout the cell cycle. This DNA can also be repaired using HDR which requires a long sequence similarity yet few complementary bases for ligating the cleaved ends of DNA. On introduction of mismatched bases in the CRISPR Cas9 construct, for restricting any further editing. However, point mutations developed due to CRISPR Cas9 gene editing must be recovered using HDR and not NHEJ. The efficiency and fidelity of the repair system can be further enhanced by keeping the upstream and downstream sequences under consideration [11]. The overall proteins involved in DSB repair are discussed further (**Table 02**).

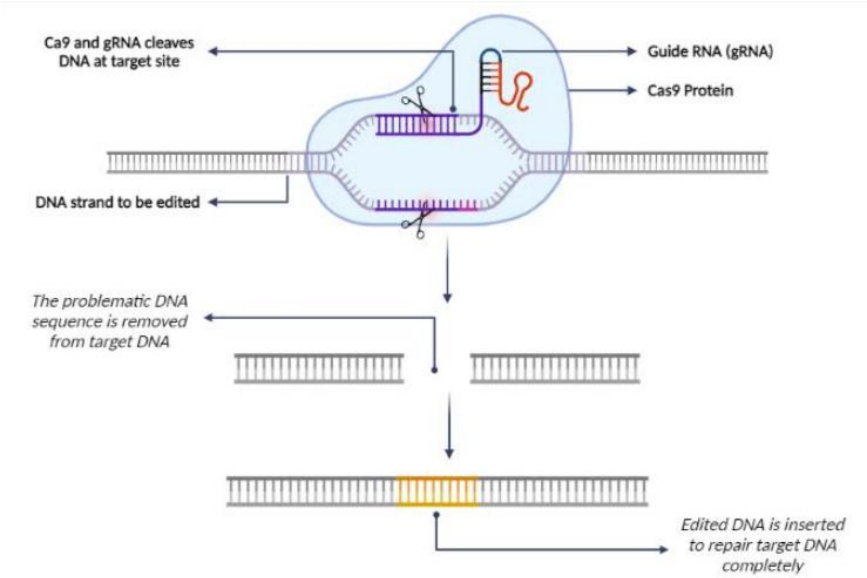

Figure 1: Basic mechanism of CRISPR-Cas9 in DNA Repair
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The most widespread Double-Strand Break (DSB) Repair pathways in eukaryotic cells are cNHEJ (Classical Nonhomologous End Joining), MMEJ (Microhomology-Mediated End Joining), SSA (Single-Strand Annealing), and HDR (Homology Directed Repair).

A. Classical Nonhomologous End Joining

The DSBs are mainly repaired when the broken DNA ends are re-ligated together through cNHEJ with minimal DNA end processing [12]. A ring-shaped protein heterodimer Ku70/Ku80 binds to the end of the double strand break. Binding of these proteins not only start the initiation of the cNHEJ, but also it prevents further resection of the DNA ends and recruits DNA-dependent Protein Kinase (DNA-PK) (***Figure 2***) [13]. DNA-PK promotes the DNA ligase IV-X-ray cross complementing group 4-XRCC4-like factor (LigIV-XRCC4-XLF) complex to ligate the both DSB ends [43]. In some cases where the Double Strand Breaks ends are not directly ligatable (***Figure 2***), some additional helping proteins should be recruited. Artemis nuclease, polynucleotide kinase, 3′ phosphatase (PNKP) and several DNA polymerases, such as Polμ and Polλ, are required for end processing to ligate the ends of non-ligatable 5’ or 3’ overhang ends of DSBs[13,14]. For Cas9-induced DSBs, Cas9 asymmetrically releases the PAM-proximal end of the cleaved DNA while the PAM-distal end of DSB remains bound to the Cas9, resulting in further processing of DSBs ends. After that, Cas9 from the PAM-distal side is removed by RNA-polymerase and facilitates chromatin transcription (FACT), although the mechanism is very unclear [13]. The specificity of Cas9 is derived from a 20 nucleotide sequences of the sgRNA complementary to a target sequence. The only restriction is that the target must have an adjacent PAM sequence (5′-NGG-3′), which occurs with a frequency of once every 16 base pairs in a random sequence [15]. Although, cNHEJ has lower fidelity and is not that much accurate, it can lead to serious DNA mutations[12]. More accurate repair pathway can be achieved through Homology-directed repair (HDR) [15].

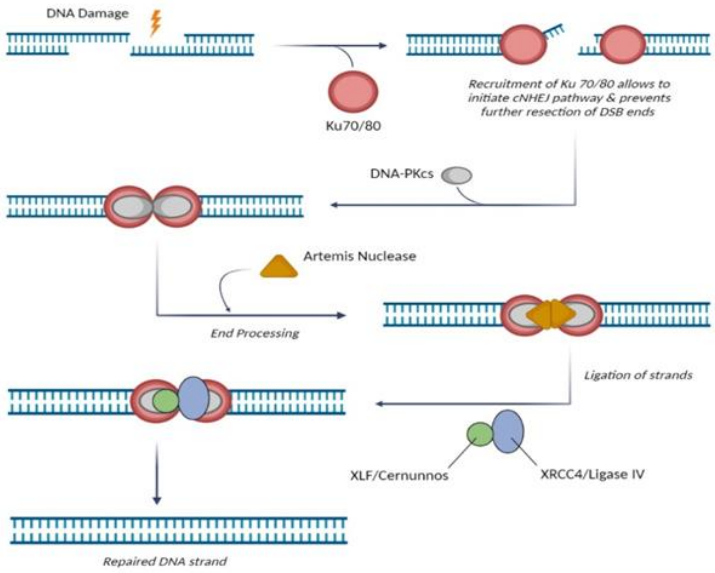

Figure 2: Overall mechanism of **classical Non-Homologous End Joining Pathway (cNHEJ Pathway)**
i. Binding of Ku 70/80 in DSB ends recruits DNA-PKcs.
ii. DNA-PKcs engage DNA Lig IV-XRCC4-XLF to ligate the DNA strands. Artemis Nuclease, Polunucleotide kinase, 3' Phosphatase (PNKP) along with Polμ and Polλ help to ligate non-ligatable DNA strands.
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B. Microhomology-Mediated End Joining

Microhomology-Mediated End Joining or MMEJ and NHEJ does not need any DNA template for the continuation of DSB repair. Rather, a short micro homologous sequence of 5 - 25 base pairs are present at the adjacent of the broken ends, that helps realignment of the DSBs. The 3' ssDNA flaps are cleaved off, which facilitates the loss of sequence information. Furthermore, the remaining gaps are filled in through DNA synthesis by the addition of complementary base sequences and the nicks are ligated by DNA Ligase III (LigIII) or DNA Ligase I (LigI) [13, 16]. The MMEJ efficiency and repair outcomes are dependent upon the microhomologies within the first 10 bp from the double-strand break. ssDNA overhangs (~20 bps) are generated during MMEJ due to some short-range end resection. This short-range end resection initiates Microhomology-Mediated End Joining [13]. In mammalian cells, the damage sensing MRN complex (MRE11-RAD50-NBS1) [17], accompanied by CtlP (C-terminal binding protein interacting protein), initiates end resection (***Figure 3***) [49, 50]. Phosphorylation of CtlP during S/G2 phase promotes the activation of MRE11 Endonuclease, which in turn generates nick at the 5' strand near to the DSB end. Nick cut at the 5' strand removes Ku proteins from the DNA ends to prevent NHEJ, so that further resection can be proceed. At this very moment, the 3′ to 5′ MRE11 exonuclease activity generates short 3′ overhangs which can be used to initiate MMEJ [13]. If the micro-homologous sequences are situated at the end of the DNA, it requires trimming. The micro-homologous sequences at the DNA ends generate heterologous 3' ssDNA flaps that are removed by XPF-ERCC1 Endonuclease (In *Saccharomyces cerevisiae* it is called Rad1-Rad10). In mammalian cells, Polθ stabilizes annealed overhangs by striping ssDNA binding-protein Replication Protein A (RPA) from ssDNA strands and the further remaining gaps are filled by DNA Polymerase [18]. The remaining nicks are sealed by DNA Ligase III (LigIII) or DNA Ligase I (LigI). The results achieved in Cas9-induced DSBs repaired through MMEJ are not totally random and can be predicated at a given DSB site. Several research shows that deletion of two or more nucleotides at the Cas9 cut site is the most common outcome. Inhibition of MMEJ decreases these nucleotide deletions significantly[13]. The ssDNA binding-protein replication protein A (RPA) prevents annealing and inhibits MMEJ, whereas poly ADP-ribose polymerase 1 (PARP1) promotes the annealing reaction by tethering DNA fragments together [13]. Some studies suggest that PARP1 promotes MMEJ by competing with Ku for DSBs end binding [19], while other studies indicate that PARP1 promotesloading of Ku Protein [20].

C. Single-Strand Annealing

Like Microhomology-Mediated End Joining, SSA also requires 3' ssDNA homologous overhangs for the annealing of the homologous sequence. The heterologous 3' flaps are needed to be removed for the continuation of Single-Strand Annealing[21]. Long-range DSB end resection is required for the initiation of the SSA pathway. Usually, Long-range DSB end resection yields long 3' ssDNA overhangs (~1000 nucleotides long) by the exonuclease activities of either EXO1 or Bloom Helicase (BLM)-DNA2 [13]. The resulting ssDNA overhangs are coated by ssDNA binding-protein replication protein A or RPA, followed by RAD52 binds to it, that facilitates the homologous annealing of two DSB ends. Various studies show that in *Saccharomyces cerevisiae*, RAD52 induces the binding of RAD51 filaments onto the ssDNA, that in turn promotes HDR. In mammals, the protein BRCA2 recruits RAD51 filament assembly, thus it inhibits SSA and favors the conditions to promote Homology-Directed

Repair [13,22**]**. Like MMEJ, XPF-ERCC1 endonuclease removes the heterologous 3' flaps in Single-Strand Annealing Pathway. However, the further details on gap filling and ligation remain unclear and are under research.

D. Homology-Directed Repair

Homology Directed Repair occurs in a particular fashion with high accuracy. It mainly uses a homologous DNA template for DNA repair. HDR proceeds with long-range end resection to form 3' ssDNA overhangs, which are then coated by RPA [23]. ATP-dependent DNA Recombinase RAD51 replaces RPA to form a long helicalfilament on the ssDNA. The 3' end of the RAD51 coated ssDNA strand is used as a primer and RAD51 aligns and pairs the ssDNA with the homologous dsDNA template for the continuation of the DSBs repair (***Figure 3***) [13].

End Resection in HDR Pathway

The End Resection in Homology Directed Repair is divided into two stages - first, MRN-CtlP mediated short-range resection; and second EXO1 or BLM/DNA2 conciliated long-range end resection.

i. Short-Range End Resection

The key protein involved in short-range end resection is MRN. The poly ADP-ribose polymerase 1 (PARP1) recruits MRN instead of Ku proteins, which may favor MMEJ, SSA & HDR. Binding of DNA-PK to the DNA ends promotes NHEJ. Whereas, the phosphorylation of Ku70 reduces the DNA binding affinity of DNA-PK, which promotes MMEJ, SSA and HDR pathways. It can also be achieved by the ubiquitin mediated Ku degradation or DNA cleavage by MRN [13].DNA-PK promotes MRN-mediated end processing, which plays a major role in the initiation of short-range end resection. A newly discovered protein called DYNLL1 (Dynein Light Chain 1 Protein) inhibits the nuclease activity of MRN [24] & ZPET (Zinc finger protein proximal to RAD18) holds up the MRN-CtlP recruitment [25]. Although, the mechanism is unknown.

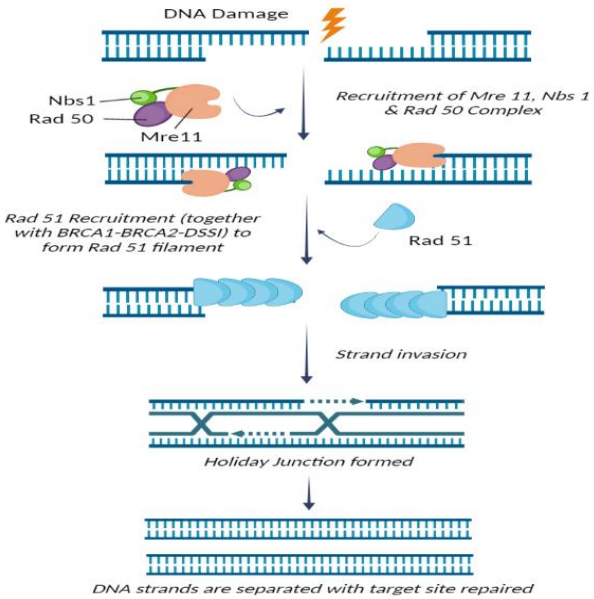

Figure 3: Basic mechanism of **Homology-Directed Repair Pathway (HDR Pathway)**
i. Rad50, combined with Mre11 and Nbs1 binds to the DSB ends and recruit RAD51 protein to form RAD51 filament.
ii. Homologous recombination occurs between both strands followed by strand invasion.
iii. DNA strands are separated with repaired target site.
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ii. Long-Range End Resection

Various enzymes BLM/DNA2 or EXO1 bind to the short overhangs generated by MRN. EXO1 has its own 5' to 3' exonuclease activity and generates 3' ssDNA overhangs, necessary for long-range resection. MRN activates EXO1, whereas CtlP inhibits it [13]. In mammalian cells, phosphorylation or dephosphorylation of RPA controls the EXO1 activity. SOSS1, the human SSB homologue 1 stimulates EXO1 mediated long-range end resection. BLM, having 5' & 3' endonuclease activity separates DNA strands, recruiting DNA2 to cleave the ssDNA. CtlP along with BLM and DNA2 activates BLM helicase and DNA2 5' endonuclease [26].

Recent discoveries shows that a regulatory mechanism by 53BP1, PTIP, RIF1, and Shieldin-CST (Shieldin complex includes SHLD1, SHLD2, SHLD3 and REV7) inhibits end resection and a competing regulatory mechanism involving BRCA1-BARD1 that promotes end resection [13]. An alternative model suggests that Shieldin complex recruits CST, Polα and Primase to double strand breaks. CST binds at the junction of dsDNA-ssDNA, which protects the 5′ end and Polα and primase execute a gap fill-in reaction [27].

RAD51 Filament Formation

During the S/G2 phase in cell cycle, RAD51 is phosphorylated by CDK1, which makes it competent against RPA that is bound with the 3' overhangs during long-range end resection (***Figure 3***). RAD51, which has recombinase activity, catalyses DNA transaction during HDR. In *S. cerevisiae*, Rad52 promotes Rad51, to form RAD51 filament on RPA-bound ssDNA (***Figure 3***). However, in humans, RAD51 filament assembly is facilitated by the BRCA2-DSS1 complex [13]. The SWI5-SFR1 complex helps in stabilizing the RAD51 bound to ssDNA.

Several negative regulatory factors remove RAD51 from ssDNA such as various helicases RECQ5, FBH1, and FANCJ in human cells. ATP hydrolysis is required to translocate along the ssDNA while removing RAD51 [28]. On the other hand, the helicase FBH1 together with SCF ubiquitin ligase complex helps in the relocation of RAD51 to cytoplasm by ubiquitination of RAD51.

RAD51 mediated Homologous Sequence Pairing

AfterRAD51 filament is formed, RAD51 along with some accessory factor proteins such as RAD54, BRCA1-BARD1, PALB2, RAD51AP1-UAF1 and HOP2-MND1 induces the interaction between the ssDNA overhangs and homologous template strand to form a heteroduplex DNA joint. BRCA1-BARD1 and RAD51AP1-UAF1 facilitate the interaction between the RAD51 filament and the homologous template dsDNA (***Figure 3***). Whereas, the RAD51 strand invasion activity is stimulated by the activation of PALB2. HOP2-MND1 helps to stabilize RAD51 filaments followed by strand invasion [13].

DNA Strand Synthesis

After the D-loop is formed, DNA Polymerase δ (Polδ), accompanied by PCNA and a clamp loader complex named RFC1 engages on the 3′ end of the invading strand, to extend the broken DNA end by using the homologous donor dsDNA as a template [13]. Further processing of the strand includes non-crossover synthesis-dependent DNA strand annealing (SDSA), double Holliday junction (dHJ) crossover and non-crossover pathway and break-induced replication (BIR) [29, 30, 31]. In SDSA, several proteins like BLM, RTEL1 or another helicase may lead to the disruption of the heteroduplex DNA joint and can be annealed with another end of the DSB, completing the repair mechanism by gap filling and ligation [13,30]. In mammals, dHJ dissolution is catalysed either by topoisomerase IIIα together with BLM-RMI1-RMI2, resulting in the formation of non-crossover products. Alternatively, dHJs can be resolved into crossover products when cleaved by nucleases such as MUS81-EME1, SLX1-SLX4, and GEN1 [13].

Table 02: Overviewon some proteins, responsible for DSB End Repair by CRISPR-Cas9:

Sl. No.	Name of the Protein	Respective Gene	Function
01.	Ku70/80 (Ku Heterodimer)	*XRCC6 & XRCC5*	Protects the DSB ends from further resection and recruits the DNA-PK (DNA dependent Protein Kinase) [13].
02.	DNA-dependent Protein Kinase (DNA-PK)	*PRKDC*	DNA-PK recruits the DNA ligase IV–X-ray cross complementing group 4-XRCC4-like factor (LigIVXRCC4-XLF) complex [42].
03.	DNA Ligase IV-X-ray cross complementing group 4-XRCC4 like Factor (LigIV-XRCC4-XLF)	*XRCC4*	Ligation of the DSB ends [43].
04.	Artemis Nuclease	*DCLRE1C*	Helps to ligate non-ligatable DNA end configurations at DSBs and is required to open the DNA hairpin intermediates in V(D)J Recombination [14].
05.	Polynucleotide Kinase 3′ Phosphatase (PNKP)	*PNKP*	Ensures that DNA termini are compatible with extension and ligation by either removing 3'-phosphate, or by phosphorylating 5'-hydroxyl groups on the ribose sugar of the DNA backbone [44].
06.	DNA Polymerase Mu (Polμ)	*POLM*	Performs gap-filling repair synthesis in the non-homologous end joining (NHEJ) [13].
07.	DNA Polymerase Lambda (Polλ)	*POLL*	Resynthesizes missing nucleotides during non-homologous end joining (NHEJ) [13].
08.	Mre11	*MRE11*	Promotes the use of HDR pathway of DNA repair between sister chromosomes when DNA damage arises [45].

09.	Nbs1	*NBS1*	Primarily involved in generating short single stranded tails [46].
10.	Rad50	*RAD50*	Maintains genome stability and cellular response towards radiation [47].
11.	Rad51	*RAD51*	A DNA strand exchange protein that forms the presynaptic complex and consequently catalyses strand invasion [48].
12.	RAD52	*RAD52*	Mediates the annealing of homologous sequences within the two DSB ends [56].
13.	C-terminal Binding Protein interacting Protein (CtlP)	*CTLP*	Initiates End Resection in conjunction with MRN complex [49, 50].
14.	ssDNA Binding-Protein Replication Protein A (RPA)	*RPA1*	Inhibit Microhomology-Mediated End Joining (MMEJ) by preventing annealing [51].
15.	Poly ADP-Ribose Polymerase 1 (PARP1)	*PARP1*	Tethers DNA fragments together and promotes the annealing reaction [52].
16.	DNA Polymerase Theta (Polθ)	*POLQ*	Strips RPA from ssDNA to promote MMEJ [18].
17.	XPF-ERCC1 Endonuclease	*XPF & ERCC-1*	Removes the resulting heterologous 3′ ssDNA flaps for microhomologies located distal to the DNA ends [53].
18.	DNA Ligase I & III	*LIG I & LIG III*	Maintains genomic integrity by joining breaks in the phosphodiester backbone of DNA that occur during replication and recombination, and as a consequence of DNA damage and its repair [54].
19.	EXO1	*EXO1*	Acts as a 5'-3' nuclease to resect DSB-ends [58].
20.	Bloom Helicase (BLM)-DNA2	*BLM*	Contributes to chromosome stability through its roles in double-strand break repair by homologous recombination and DNA replication fork restart during the replication stress response [55].
21.	BRCA2	*BRCA2*	Promotes RAD51 filament assembly in mammals [57].

USE OF CRISPR BASED ASSAY TO STUDY DNA REPAIR IN SPACE TRAVELERS

Microgravity conditions have been shown to influence the choice of DNA repair method by CRISPR Cas9. However, due to safety concerns and technological limitations, these studies have frequently relied on the generation of DSBs on Earth, accompanied by the freezing and sending of biological material to space to assess DNA repair choices in microgravity. The recognition of the DNA break and the assembly of DNA repair proteins at the break site are thought to be critical determinants of repair pathway choice and may happen rapidly after the DSB. As a result, it's possible that the decision of repair happened on Earth rather than in space in prior studies of DNA repair in space. The Earth has several magneto atmospheric layers that often induce damage in DNA of the space explorers who cross these layers. Exposure to radiations of different wavelengths as well as high energy molecules results in induction of small to extensive large scale physiological and genetic damage. These changes are usually in nature[32].

The galactic radiation is extremely damaging and the space craft materials are not enough to protect the space explorers against those drastic effects. Exposure to light flashes during moon ventures in turn expose the retina of astronauts to harmful charged particles that constitute a large part of these radiations resulting in early occurrence of cataracts [33]. In order to repair such damaging effects on the space traveller's genetic material, the CRISPR Cas9 is a promising tool that has the power to edit genomes prone to such harmful ionizing radiation. The CRISPR-based mutagenesis methodology is used to generate double-stranded breaks at a characterized genomic locus [34].
The mechanism of repair pathways was described in the previous sections of our review. A CRISPR based assay was studied by a group of high school students. They primarily focused on the ability of CRISPR Cas9 to generate double strand breaks in higher organisms. The first genetic transformation and CRISPR Cas9 genome editing in space was first demonstrated by this research group emphasizing on the fact that CRISPR Cas9 can be an efficient molecular biology tool kit choice for repairing DNA in the International Space Station [32].

CRISPR Cas9 does not use radiations and reagents for repairing the DNA damaged during space travel and most importantly the DSBs are location specific and editing of this DNA sequence for repairing purposes by methods listed by the International Space station is relatively much easier and hassle free[34]. The transformation of

Saccharomyces cerevisiae with the aid of exogenous genetic material was the first model for utilization of CRISPR Cas9 systems in space. A simulated environment comprising of microgravity and generation of DNA lesions and repairing these lesions using molecular biology tools was the main component of the experiment Despite the difficulties in creating the exact conditions required to reproduce outer space conditions These conditions laid strong foundations for future investigations based on space ventures. Other than developing several assays for determining the mechanism of DNA repair in space travellers, CRISPR Cas9 systems aims to help explorers in determining the requirement of a radiation shield in a particular environment. Wallace et.al studies have been extremely beneficial in studying the crew's exposure to radiations as well as planning safety in advance. Instead of relying on the samples sent up to the space station from Earth, scientists can analyse the DNA that sustained the damage in the space environment itself; however, the conditions required for CRISPR Cas9 has to be customized for its proper functioning in the outer space environment. As per Wallace et al., more research is required to establish the use of CRISPR Cas9 systems and their use in outer space and potential avenues are yet to be explored to set up a self-customized molecular biology laboratory in the International Space Station [35].

FUTURE ASPECTS OF GENOME EDITING BY CRISPR CAS 9 BEYOND SPACE.

Gene editing and its rapid delivery in several model organisms like Drosophila, Zebrafishes, plants and mice has certainly opened wide opportunities to explore diverse uses of gene editing in the near future. For instance, the dCas9 produced when nuclease activity of the CRISPR-Cas 9 system is deactivated by mutations, the dCas 9 system is shaped. The CRISPR-dCas9 based artificial transcription factors (ATFs), consisting of DNA-binding domain (DBD), have been shown useful for cancer therapy because it has potential in DNA manipulation for target gene modification without causing DNA double strand cleavage and also in tumour resistance mechanism. Combining CRISPR Cas9 and ATFs is considered to be therapeutically beneficial[36]. Other uses include treatment of chemoresistance, drug opposition, epigenetic guidelines and immune resistant guidelines in breast cancer cells, adenocarcinoma and melanoma cells hence showing a promising approach for cancer therapy. The CRISPR Cas9 mechanism of gene editing also finds its use in agriculture where the nutritional value can be increased or potential resistance to pests and drought can be decreased via editing genes. For instance, scientists have used CRISPR-Cas as a biotechnological tool to modify a corn gene ARGOS8 converting it into a drought resistant strain. Though this trial was effective in improving drought resistance. However, yield was low due to poor levels of gene expression. So, scientists removed the native promoter of that gene and incorporated a new promoter[15].

Gene editing using CRISPR Systems have been proven extremely useful for treating hereditary disorders such as Haemophilia, Alzheimer's, Parkinson's, Cystic fibrosis, Sickle cell anaemia and many more uncountable diseases are still under investigation [37].

CONCLUSION

Though CRISPR based therapies have several advantages, there are ethical questions that are yet to be answered. The ongoing trials and methods of utilizing CRISPR Cas9 in several fields of molecular biology will be indeed a great accomplishment and the already existing preliminaries have already helped us to depict the use of CRISPR as one of the most successful genome editing tools. Coupling with several combinatorial therapies, CRISPR Cas9 systems will indeed play a major role in the near future.

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