Imagine being able to go into a human cell and snip out a defective sequence of DNA and then replace it with the correct sequence in order to cure a genetic disorder or disease. Since the discovery of the CRISPR-Cas9 system this idea is no longer just a wishful dream for scientists. In fact, it is growing closer to being a reality with each passing day. CRISPRs (clustered regularly interspaced short palindromic repeats) are sections of DNA found in bacteria and archaea that contain repeating base sequences in short segments. Each base sequence repetition is followed by "spacer DNA", which are short segments that are created when a prokaryote encounters a plasmid or virus. Cas9 (CRISPR associated protein 9) is a DNA endonuclease enzyme that is guided by RNA. It is specifically associated with the CRISPR segments of DNA. Together the CRISPRs and Cas9 form a system that aids in the immune defense of prokaryotic cells. Cas9 is able to recognize and cleave foreign DNA from viruses and plasmids rendering them useless. The process is similar to how RNA interference (RNAi) functions in eukaryotic organisms. Recently, research in this particular field has been expanding at an exponential rate.

When the CRISPR-Cas9 system was first discovered it was initially only recognized as being a part of bacterial adaptive immunity. As further research was carried out, however, scientists realized that these particular endonucleases could be used for genome editing and regulation of the transcription process in eukaryotic organisms as well as prokaryotic organisms. The Cas9 endonuclease allows for extreme flexibility when it comes to targeting and cleaving a specific sequence of DNA within a genome. Cas9 is able to be programmed using specific RNA sequences that act as guides for the endonuclease and 'tell' it which DNA sequence to target and cleave. The endonuclease breaks both strands of the DNA which can then be repaired by non-homologous end joining. For the regulation of transcription, a de-activated form of Cas9 is used. On the one hand, the de-activated Cas9 does not allow RNA polymerase to bind to the promoter sequences needed to initiate the transcription process thus inhibiting the rate of transcription. On the other hand, RNA polymerase can be recruited to the transcription initiation site when the de-activated Cas9 is bound to transcriptional activators which leads to transcription being promoted. CRISPR has also been used to study the evolution of bacteria and bacteriophages. CRISPR loci have the ability to differentiate at a rapid rate when they are exposed to viruses insuring that the bacteria are able to maintain a high level of immunological defense. However, it has also been discovered that bacteriophages are able to target and use the CRISPR-Cas9 system against bacteria themselves. This discovery has led to the conclusion that the CRISPR-Cas9 system plays a large role in bacterial and viral evolution (Barrangou,R., 2015).

Virology is an area in which the CRISPR-Cas9 system is being actively applied and used for research purposes. In one study, the CRISPR-Cas9 system was used to engineer herpes simplex viruses in order to allow scientists to be able to study their pathogenicity more efficiently. The herpes simplex viruses are an important set of viruses in terms of medicine due to the fact that they may be able to be used as a recombinant vaccine vector for cancer (Russell et al., 2014).

Researchers were trying to create recombinant strains of herpes simplex virus using CRISPR-Cas9 to target a specific location within the virus' genome between the positions U_L26 and U_L27. A position in this specific area of the genome was necessary because foreign genes can be introduced in that region without causing the viral cell to lose its virulence. CRISPR-Cas9 was a perfect candidate for this particular research due to the fact that it is able to target areas of the genome so specifically. Overall, the CRISPR-Cas9 was highly successful at yielding high rates of recombinant herpes simplex viruses as compared to other transfection/infection methods (Russell et al., 2014).

Another study involved using the CRISPR-Cas9 system to effectively inhibit the replication of the hepatitis B virus and therefore preventing chronic infection (Dong et al., 2015). Treatments that are currently available for the hepatitis B virus are unable to render cccDNA useless and thus infection is allowed to progress within the host. CRISPR-Cas9 however has the ability to directly cleave this cccDNA. Single strand guide RNAs were used to direct the Cas9 endonuclease to the appropriate target site within the virus' genome. Once CRISPR-Cas9 had interfered with the cccDNA of the hepatitis B virus testing of the mice used in the studied had lower levels of cccDNA and the hepatitis B virus protein (Dong et al., 2015).

The hepatitis C virus can also be rendered useless and no longer be able to replicate within host cells using CRISPR-Cas9 as a defense. (Keener, A., 2015). The Cas9 enzyme used to bind hepatitis C virus is found in the bacteria *Francisella novicida*. This particular version of the endonuclease (FnCas9) is a bit of a rebel when compared to other Cas proteins. Instead of cutting up RNA FnCas9 it binds the RNA within the cytosol. This binding basically keeps the RNA from being copied or translated into viral proteins thus inhibiting replication of the virus (Keener, A., 2015).

CRISPR-Cas9 is also being used to correct mutations within the genome that cause genetic diseases and disorders. Duchenne muscular dystrophy is one such disease that has shown promise for this type of treatment using CRISPR-Cas9 (Hongmei et al., 2015). In most cases of Duchenne muscular dystrophy there is a deletion of exon 44. In order to restore this exon (and thus restoring the proper amino acid sequence) CRISPR-Cas9 was used. Induced pluripotent stem cells from patients with Duchenne muscular dystrophy were used to see if the dystrophin protein could be restored by genetic correction using CRISPR-Cas9. Three different correction methods were performed during the study using CRISPR/Cas9 to assess the risk of off-target mutagenesis: exon skipping, frameshifting, and exon knockin. A problem that is faced when treating genetic diseases with gene therapy is off-target mutagenesis, however, CRISPR-Cas9 lowers the risk for this dramatically- making it a candidate for gene therapy treatment. The target site for the CRISPR single stranded guide RNAs was identified at the $5'$ region of exon 45 in the dystrophin gene (Hongmei et al., 2015). The dystrophin protein reading frame lacking exon 44 was identified. CRISPR-Cas9 was then able to restore the reading frame by disrupting

the splicing acceptor. Overall, the use of CRISPR-Cas9 was successful in restoring the dystrophin protein in the induced pluripotent cells (Hongmei et al., 2015).

CRISPR-Cas9 is even being used to try to restore extinct species. In March of this year, scientists at Harvard used CRISPR-Cas9 to cleave DNA segments that coded for certain mammoth characteristics such as ear size and hairiness. (Crew, B., 2015). They then used CRISPR-Cas9 to insert these particular genes into the genetic code of an elephant. However, all of this was done in a petri dish and not in an actual, viable elephant egg. (Crew,B., 2015). Maybe in a few years it will be possible to insert mammoth genes into an egg and create an elephant with mammoth genes in its genetic code.

Perhaps the most controversial use of CRISPR-Cas9 is using the system to modify the genome at the germline level. Earlier this year renowned biologists from around the world met to discuss the ethics behind this possibility (Wade, N., 2015). Using CRISPR to change germline cells would not only affect the organism itself but also any offspring that it might produce. This type of change in the human germline could control human heredity (Wade, N., 2015). At the meeting those participating asked that no one experiment with the CRISPR-Cas9 system in terms of the human germline. However, only a month later a group of scientists in China published a paper stating that they had used CRISPR-Cas9 to alter human zygotes (Liang, et al. 2015). In this study, CRISPR-Cas9 was used to try to correct a genetic disorder called beta-thalassemia by correcting a mutation in the beta globin gene. The human embryos that were used "tripronuclear" embryos meaning two sperm have delivered their DNA into a single egg- this occurs sometimes during in vitro fertilization. 86 embryos were used in the study of which only 71 actually survived long enough to be studied. The study resulted in both cells that had suffered off-target mutagenesis and mosaic cells in other words the experiment failed. Suffice it to say that modification of human embryos is not ready for clinical trials at this point in time (Liang, et al. 2015) Perhaps in the future this technique will be perfected and scientists will be able to prevent a disease in an embryo before it even occurs.

The CRISPR-Cas9 system shows great promise in many different areas of research from understanding microbial evolution to treatment of viral diseases and genetic diseases to manipulation of embryos and being able to change genetic information at the germ cell level. Its ability to perform so many versatile and yet specific tasks will most likely ensure that it stays at the forefront of the scientific frontier for a long time. CRISPS-Cas9 just might be the future in the world of medicine and genetics.

Works Cited:

Barrangou, R.: The roles of CRISPR-Cas systems in adaptive immunity and beyond. *Current Opinion in Immunology* 2015, 32:36-41

Crew, Bec. 2015. http://www.sciencealert.com/mammoth-dna-has-been-implanted-into-functioning-elephant-cells

Dong, C., Qu, L., Wang, H., Wei, L., Dong, Y., Xiong, S.: Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. *Antiviral Research* 2015, 118:110-117.

Hongmei, L., Naoko, F., Noriko, S., Saya, S., Tokiko, O., Tetsushi, S., Michihiro, T., Naoki, A., Akira, W., Hidetoshi, S., Takashi,Y.: Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9. *Stem Cell Reports* 2015, 4:143-154.

Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., Lv, J., Xie, X., Chen, Y., Li, Y., Sun, Y., Bai, Y., Songyang, Z., Ma, W.: CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. http://link.springer.com/article/10.1007/s13238-015-0153-5/fulltext.html

Keener, A. 2015. http://www.the-scientist.com/?articles.view/articleNo/42827/title/Combatting-Viruses-with-RNA-Targeted-CRISPR/

Russell, T., Stefanovic, T., Tscharke, D.: Engineering Herpes simplex virus by infection-transfection methods including recombination site targeting by CRISPR/Cas9 nucleases. *Journal of Virological Methods* 2014, 213:18-25.

Wade, N., 2015. http://www.nytimes.com/2015/03/20/science/biologists-call-for-halt-to-gene-editing-technique-in-humans.html?_r=0