

Introduction

The Arctic region is undergoing rapid and unprecedented changes due to climate change. As the Arctic ice melts, revealing previously inaccessible areas, scientists are discovering a treasure trove of ancient secrets. Among them are the mysterious Pandoraviruses, giant viruses that have lain dormant in the ice for thousands of years. These ancient germs pose intriguing questions about the potential risks they may pose to human and environmental health. In this article, we delve into the world of Pandoraviruses and explore the concerns surrounding their release in the context of a warming Arctic.

Ancient Giants:

Pandoraviruses are a group of large viruses that were first discovered in 2013. They are among the largest known viruses, characterized by their enormous size and complex genetic makeup. These viruses harbour an extensive collection of genes, some of which were previously unseen in the viral world. Due to their unique characteristics, Pandoraviruses have captured the attention of scientists worldwide.

The Thawing Arctic: Unleashing Primordial Germs:

Climate change is causing the Arctic to warm at an alarming rate. As a result, permafrost, the frozen ground that has preserved ancient organisms for thousands of years, is thawing. Within this frozen ground lies an abundance of ancient microbial life, including viruses like the Pandoraviruses.

The concern arises when these viruses become exposed and potentially reactivate as a consequence of the melting ice.



The Risks and Uncertainties:

When it comes to ancient viruses, there is still much that remains unknown. Scientists are grappling with questions regarding the potential risks associated with the release of Pandoraviruses. Some concerns include their ability to infect and cause harm to living organisms, including humans. However, it is important to note that while Pandoraviruses are capable of infecting amoebas, their pathogenicity to humans is not yet fully understood.

The Complexity of Host Specificity:

One reassuring factor is the concept of host specificity. Viruses typically exhibit specificity for certain host species, and Pandoraviruses have shown a preference for amoebas. This suggests that even if they were to become active, their ability to infect and harm human beings might be limited. However, further research is necessary to fully comprehend the extent of their host range and potential risks.

Environmental Impact:

The release of ancient viruses could have ecological consequences. As they interact with current microbial communities in the Arctic, they may disrupt the delicate balance of existing ecosystems. This, in turn, could impact the flora and fauna of the region, including vulnerable species that rely on these ecosystems for survival.

Mitigating the Risks:

To address the concerns associated with the release of ancient germs, scientists are working diligently to study and understand these viruses. Through ongoing research, the researchers also investigated the role of the major capsid protein (MCP) in Mollivirus, a phylogenetically related virus to Pandoravirus. They found that MCP was essential for virion formation in Mollivirus, despite its ovoid shape. Genetic deletion of the MCP gene using CRISPR/Cas9 resulted in impaired virion biogenesis, indicating a scaffolding function for MCP.

With that said, why are giant viruses of interest to the scientific community?

Giant viruses (GVs) have captivated scientists since their discovery due to their unusually large genomes and viral particles that rival the size of some cellular organisms. These viruses possess unique features, such as encoding proteins involved in translation and energy metabolism, which are typically associated with cellular organisms. The origin of GV remains a topic of debate, with two main theories proposing reductive evolution from an ancestral cell or evolution from smaller viruses. To unravel the mysteries surrounding GV, researchers have focused on their genome diversity but neglected their functional genomics.



In a 2023 Nature paper, a team of scientists presents an experimental framework using CRISPR/Cas9 to genetically modify nuclear GV and their host, *Acanthamoeba castellanii*. The study provides insights into the evolution from small icosahedral viruses to amphora-shaped GV and uncovers a core essential genome in Pandoravirus, shedding light on the genetic basis of viral gigantism.

How did the researchers use CRISPR/Cas9 to unveil the secrets within the Pandora Virus?

The researchers developed a CRISPR/Cas9 system to genetically manipulate the host organism, *Acanthamoeba castellanii*, and successfully modified the host's DNA. They demonstrated the efficiency of this system by targeting specific genes and observing a significant decrease in the expression of the targeted genes. The researchers also designed gRNAs to target the cellulose synthase genes in *Acanthamoeba castellanii* and achieved complete modification of these genes using Cas9.



Next, the researchers investigated the capability of Cas9 to target the genomes of different GVs. They found that nuclear GVs, such as *Pandoravirus neocaledonia* and *Mollivirus kamchatka*, could be efficiently targeted and their replication inhibited by Cas9. However, Cas9 was unable to target strictly cytoplasmic viruses like *Pithovirus sibericum* and *Mimivirus reunion*. The researchers hypothesized that the shielding of the viral DNA in cytoplasmic viruses might prevent Cas9 from accessing the genome.

To study the essential genes in *Pandoravirus*, the researchers performed a genetic screen using CRISPR/Cas9 and targeted various genes throughout the viral genome. They observed that the fitness reduction of *Pandoravirus* varied depending on the targeted gene's location. Targeting genes at the 5' end of the genome resulted in a significant decrease in viral fitness, while targeting genes at the 3' end led to large deletions in the genome. This suggested that *Pandoravirus* might have limited efficiency in repairing double-strand breaks at the 3' end.

Further analysis revealed the existence of a core essential genome located at the 5' end of the *Pandoravirus* genome. This core region contained genes that were conserved in related viruses from the family *Phycodnaviridae* and the virus *Mollivirus*. In contrast, genes at the 3' end of the genome were less essential and tended to accumulate large deletions. The findings supported the hypothesis of genome expansion in *Pandoravirus* and provided evidence for the transition from a *phycodnavirus*-like ancestor to the larger *Pandoravirus*.

The researchers also investigated the role of the major capsid protein (MCP) in *Mollivirus*, a phylogenetically related virus to *Pandoravirus*. They found that MCP was essential for virion formation in *Mollivirus*, despite its ovoid shape. Genetic deletion of the MCP gene using CRISPR/Cas9 resulted in impaired virion biogenesis, indicating a scaffolding function for MCP.

So, what does this all mean?

This study demonstrates the successful application of CRISPR/Cas9 technology for genetic manipulation of both host organisms and giant viruses. The findings provide valuable insights into the evolution and functional genomics of GVs. The existence of a core essential genome in Pandoravirus supports the hypothesis of genome expansion and highlights the importance of conserved genes in viral replication. The analysis of gene distribution and essentiality in Pandoravirus suggests an evolutionary pathway from smaller viruses towards giant viruses, with genetic redundancy increasing as a result of genome expansion.

Furthermore, the study reveals the essential role of the major capsid protein in virion formation in Mollivirus. This finding supports the hypothesis that the ovoid shape of Mollivirus might be a trace of its icosahedral ancestry, and that MCP function has been replaced during evolution.

The development of genetic tools for studying GVs opens up new avenues for research on these enigmatic viruses. By manipulating their genomes, scientists can uncover conserved biological processes, host-pathogen interactions, and the adaptive mechanisms that contribute to viral gigantism. This study represents a significant step towards understanding the origins and evolutionary history of GVs, shedding light on the complex relationship between viruses and their hosts.

Conclusion:

While the discovery of ancient viruses in the melting Arctic may sound alarming, it is essential to approach the topic with scientific rigor and balanced perspective. While the release of Pandoraviruses raises questions about their potential risks, the current understanding suggests that the immediate threat to human health seems limited. The genetic modification of giant viruses using CRISPR/Cas9 provides a powerful tool for investigating their genomes and unraveling their evolutionary origins. The paper published in Nature contributes to our understanding of viral gigantism and the unique features of giant viruses, offering new perspectives on the coevolution of viruses and their hosts. Further research in this field holds great potential for uncovering the intricate mechanisms and evolutionary pathways that have shaped the viral world.

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