Dr Alex Borodavka explains his research investigating the molecular interactions between genomic RNA segments in rotaviruses, and how this work could contribute to the future design of novel vaccines.
Researchers at the Universities of Leeds and York in the UK, as well as the Ludwig-Maximilian University of Munich, Germany, are examining how segmented RNA genetic material is able to consistently organise and package itself in rotaviruses. Their findings could help to develop novel treatments for a range of RNA viruses.

Rotaviruses are highly contagious pathogens that mainly infect children. Most children will be infected at least once by the age of five, and that means there are a huge number of cases every year – estimated at over 114 million – with upwards of 200,000 deaths. Infection with a rotavirus can cause diarrhoea and vomiting, often severe if basic treatment is not available. As the biggest determinant of survival is access to appropriate medical care, these pathogens pose the biggest problems in poorer countries where such essentials are not necessarily a given. Currently, there are no drugs that can treat rotavirus once it has infected someone. However, there are several commercially available vaccines. Since their development, hospitalisations caused by rotavirus have dropped dramatically worldwide, but more could certainly still be done in terms of treating the disease.

In order to better understand how to combat the virus, it is essential to understand its molecular underpinnings. Viruses in general contain genetic information (either RNA or DNA) surrounded by a protein shell. The shell protects the genetic information from destruction and helps it to enter host cells. At that point, the virus’ genetic material comes into play, hijacking the host’s protein machinery to make many copies of itself. These copies will then acquire a new protective shell during a process termed ‘genome packaging’, and will burst out of the cells to continue infecting more cells. Rotaviruses code their genetic material using double-stranded RNA, which is divided into 11 individual segments. This genome segmentation is one of the great unknowns when it comes to rotaviruses. In particular, understanding how the rotavirus is able to package exactly 11 segments into a virus particle is a key challenge. Most viruses only have a single molecule of either DNA or RNA, which is relatively straightforward to replicate. What Dr Alex Borodavka and his collaborators at the Universities of Leeds and York, UK, and the Ludwig-Maximilian University of Munich, Germany, want to find out is how the 11 segments of a rotavirus are successfully replicated. ‘During replication, RNA segments are copied many times. It is not clear how rotaviruses ‘count’ up to 11 so that each new virus acquires a single copy of each segment,’ he explains.

SENSITIVE METHODS
It is very difficult to describe the precise molecular interactions that govern the assortment of the RNA segments. Borodavka and his colleagues are utilising a few exceptionally sensitive techniques to investigate the mechanisms in question, all...
of which fall under the broad categorisation of single molecule fluorescence (SMF) techniques.

Primarily, the research team is harnessing the power of fluorescence cross-correlation spectroscopy (FCCS) to investigate the intermolecular interactions that may be involved in the genome packaging in rotaviruses. This method allows detection of the interacting, fluorescently labelled molecules in a sample. Borodavka explains the advantages of FCCS for his work: ‘Owing to its high sensitivity, this technique is particularly suitable for interrogating specific, stable inter-segment RNA-RNA interactions at sub-nanomolar concentrations. Such an experimental approach avoids common problems associated with non-specific aggregation, particularly in the presence of multiple RNA-binding proteins and RNA molecules.’

In addition to microscopic techniques, Borodavka is also applying aptamer-based technologies to probe the RNA interactions involved in rotavirus assembly. RNA aptamers are small RNA molecules that can bind a target sequence with high affinity. These small RNA sequences are identified using an experimental approach known as ‘systemic evolution of ligands by exponential enrichment’, or SELEX. This technique involves the generation of a large library of potential RNA aptamers that are then put through a rigorous selection process in order to procure the RNA sequences that bind with the highest specificity to the target. These methods are used to identify the specific sites of interactions involved in inter-segment interactions in segmented RNA viruses.

**MAPPING INTERACTIONS**

The project has already yielded some very promising results. Borodavka’s team has been able to confirm previous indications that complex RNA-RNA interactions between segments play a significant role in the selection and organisation of the 11 segments. The team has also identified a mechanism through which the viral non-structural protein NSP2 can stabilise inter-segment contacts during rotavirus replication. Upon binding the RNA, this protein remodels the single-stranded RNAs, resulting in the formation of new RNA-RNA contacts between the single-stranded precursors of double-stranded RNA genomic segments. These changes of conformation in the RNA segment precursors create the contact points necessary to correctly assemble all 11 disparate RNA molecules prior to packaging into one viral capsid. These findings represent a vital breakthrough in our understanding of how rotaviruses can package exactly one copy of each segment into their capsids.

While the work is ongoing, these results have brought the team very close to answering the important question of how rotaviruses are able to package their genomic information with such consistency. In order to complement and further expand on these findings, Borodavka hopes to use the recently developed reverse genetics system for rotaviruses to gain novel insights into the mechanisms of genome packaging in rotaviruses, and potentially other segmented RNA viruses. Borodavka explains how reverse genetics should give greater insight into the assembly process: ‘Using this approach, we can now test how disruption of the identified inter-segment interactions affects replication and assembly of rotaviruses in cells. We also anticipate that these results will help to further understand the rules that control packaging of multiple RNA segments in rotaviruses, so that their genomes could be manipulated to create new vaccines, viral oncolytic agents and RNA delivery systems.’

**BROADER IMPACT**

Borodavka’s project is not only making novel and fascinating findings regarding the basic molecular biology of rotavirus, it is also likely to prove essential in the understanding of a whole host of other segmented RNA viruses. Influenza is one such segmented virus and, in its case, it is established that gene assortment is an important mechanism involved in the virus’ ability to pose multiple challenges to vaccinations. Consequently, it is likely this work will open new avenues for the development of anti-viral treatments for several different viruses that currently cause major healthcare problems across the globe. It is clear that these researchers are undertaking essential work into the organisation and replication of rotavirus genomes, as Borodavka himself explains: ‘The novel mechanistic insights gained into the molecular basis underlying inter-segment interactions in rotaviruses will open up unprecedented opportunities for delineating complex molecular interactions that govern assembly of other segmented RNA viruses.’

What’s more, by getting to grips with the network of inter-segment interactions in rotaviruses, researchers will be able to accelerate efforts to rationally design new vaccines that have higher success rates for these viruses. It may also yield potential targets for antiviral drugs, thus addressing a significant unmet need.