Impact Objectives

- Understand the molecular basis of the replication and packaging of 11 distinct RNA segments in rotaviruses
- Contribute to the development of new treatments against rotaviruses

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

Can you begin by explaining why RNA viruses are so difficult to deal with?

As part of my initial PhD training I started investigating the molecular mechanisms of RNA virus assembly. Our research project was focused on the long-standing issue of selective genome packaging in single-stranded (ss)RNA viruses, which constitute one of the largest groups of human and animal pathogens. The error-prone replication of RNA viruses poses multiple challenges to the development of vaccines and efficacious anti-viral drugs. A step change in our understanding of viral life cycles at a molecular level will help us to identify new therapeutic strategies and drug targets. Recently, we were able to gain novel insights into the roles of viral ssRNAs in the mechanisms of infectious virus assembly. The success of our studies at the University of Leeds, UK, encouraged me to continue investigating viral RNAs, including those of viruses with segmented RNA genomes, such as rotaviruses and other reoviruses.

What are the key knowledge gaps when it comes to rotaviruses?

One of the big questions in understanding replication of segmented viruses, including rotaviruses and influenza viruses, is how these viruses non-randomly package multiple genome segments. We don’t know to what extent different virus strains can reassort their gene segments with each other, and we also don’t know whether individual genome segments are more important in the genome packaging process – that is, playing the role of a ‘master’ segment that controls gene assortment. Answering these questions would open new avenues for improved disease control strategies, and also shed light on the evolution of this important class of pathogens.

What is the main goal of your research?

Despite the importance of understanding the molecular basis of segmented RNA genome packaging, there is very little knowledge of how 11 distinct ssRNAs are selected in rotaviruses. Our goal is to find out how rotaviruses select the right gene segments by exploring their individual structures and how they interact with each other. Answering these questions will bring us closer to the development of new treatments and a rational design of vaccines against rotaviruses.

There are already vaccines in use for rotavirus, so why is this research necessary?

Most children who receive the currently available vaccines will be protected from severe rotavirus illness. However, there are remaining concerns about the efficacy of the existing vaccines in developing countries. There is also the possibility of new antigenically and genetically distinct strains emerging in countries where rotavirus vaccinations are in place, which might result in lower vaccine effectiveness. Understanding the mechanisms of gene selection and segment assortment in rotaviruses will thus provide new insights into the evolution of these pathogens.

Future development of any rotavirus vaccine and improvement of the currently licensed vaccines are still hampered by our limited knowledge of the mechanisms of segment reassortment in rotaviruses.

How do you see your work progressing in the future?

Despite significant progress in understanding the structural basis of rotavirus replication, there is still no detailed understanding of how these viruses select their RNA segments. Recent advances in single molecule fluorescence imaging and RNA structure probing will likely yield novel data, shedding light on this fundamental problem in virology. More importantly, the recent development of an entirely plasmid-based reverse genetics system for rotaviruses earlier this year has marked an important milestone in our studies of these pathogens. The reverse genetics system for rotaviruses will allow us to finally test the functional relevance of our in vitro findings and to map the entire RNA genome interactions network in rotaviruses.
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 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.