Learning from the best

Dr Jeffrey Kieft, from the Department of Biochemistry and Molecular Genetics at the University of Colorado School of Medicine, is using studies of viral RNA to discover not just how viruses cause illness, but also to develop our understanding of fundamental cellular processes.

RNA stands for ribonucleic acid – it is a vital molecule involved in turning the information stored in DNA into useful proteins, as well as in many other critical biological processes. With a similar construction to DNA of long chains of nucleotides (a nitrogenous base coupled with a ribose sugar and phosphate), it is created, translated and degraded by cellular 'machinery' as necessary to maintain cellular functions. Viruses exploit this system by inserting their own RNA into a cell to hijack the cell and turn it into a factory for viral replication, usually with the result that the cell ruptures and releases new viral particles to infect others.

The Kieft lab are interested in how this versatile molecule, which can encode information like DNA as well as forming complex and compactly-folded bioactive structures like a protein, is able to exert its effects. They are able to draw on a rich diversity of available RNA for their studies; it is thought there may be more than one billion species of virus with only a small proportion so far identified by science. Those discovered exhibit myriad functional characteristics, fine-tuned through evolution for subtlety and elegance. The team has a dual strategy, to examine the virus in search of therapeutic targets at the same time as using viral RNA as a tool to probe fundamental elements of RNA-based cellular processes.

The first is important enough by itself, viral diseases place a huge burden on healthcare systems around the world. The flavivirus (FV) family, which includes Yellow Fever (*flavus* is Latin for yellow), West Nile Virus and Dengue Fever (DENV), is a major focus of their work. In 2010 these viruses caused over 100 million symptomatic human infections. These are often severe and debilitating, and in some cases fatal. They are also widespread, with the World Health Organization estimating that over 40% of the global population were at risk in 2014. International trade and climate change threaten to further extend the range of the mosquito vector which transmits the disease, and with no effective therapies or vaccines against the majority of FVs, further research is urgently needed.

TEACHING US A LESSON

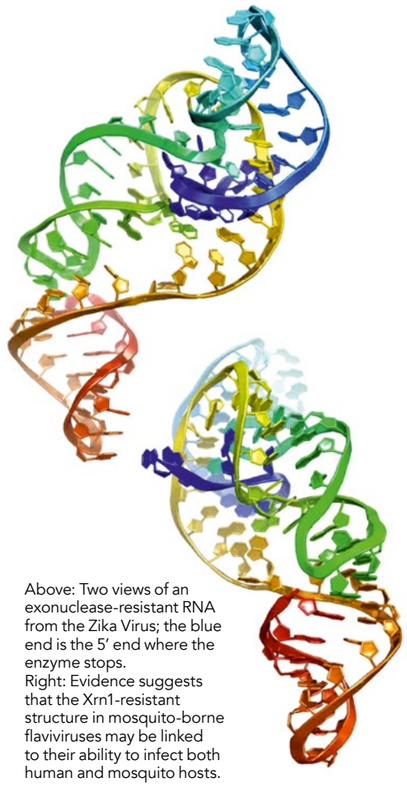
FVs gain entry to the cell by receptor-mediated endocytosis (essentially piggy-backing onto cell signalling mechanisms). This releases their genomic RNA (gRNA) into the cell where it is translated as if it were part of the cell's own RNA. This makes a single protein which is further divided to make all the necessary elements for viral replication and transmission. In order to regulate the way that proteins are created within a cell, it has to be possible for normal cellular RNA to be switched off. Cells do this by degrading the RNA once its job is done, but this poses a problem for FVs which need to replicate and protect their RNA to make more copies of themselves.

Dr Kieft and his team have shown how DENV uses a unique RNA structure in a particularly elegant way of overcoming this problem. The exoribonuclease Xrn1 (a cellular enzyme which degrades RNA) chews its way through the gRNA in a specific direction known as 5'–3'. However it stops short of degrading the entire strand, leaving a section dubbed a subgenomic flaviviral RNA (sfRNA). This remaining section is associated with many of the cytotoxic (cell damaging) effects of the virus, in part by inhibiting the cell's anti-viral defences.

LEARNING THE HARD WAY

The team developed an assay to confirm that specific folding patterns in the RNA, related to nucleotide sequences in the untranslated region which remains after degradation, are responsible for stopping Xrn1 in its tracks. They identified a complex three-dimensional fold, created by the pairing of complementary nucleotides on a single strand, which creates a remarkable barrier to Xrn1. Because Xrn1 moves from the 5' to the 3' end of the strand (each end is named

The team has a dual strategy, to examine the virus in search of therapeutic targets at the same time as using viral RNA as a tool to probe fundamental elements of RNA-based cellular processes”



Above: Two views of an exonuclease-resistant RNA from the Zika Virus; the blue end is the 5′ end where the enzyme stops.
Right: Evidence suggests that the Xrn1-resistant structure in mosquito-borne flaviviruses may be linked to their ability to infect both human and mosquito hosts.

after the exposed carbons on the ribose ring which confer directionality to nucleic acids), the barrier it finds when degrading the RNA is not encountered by the virus' own polymerase which makes copies from the other direction.

Further work by Dr Kieft and his collaborators has shown that this is not restricted to DENV: the sequences necessary to create the interfering structures are conserved across the mosquito-borne flaviviruses. They have shown that a very similar structure is present in the Zika virus RNA, which has recently been the subject of significant media attention for its effects, and that this also results in production of sfRNA.

KNOWLEDGE IS POWER

For RNA viruses, hijacking of the host's translational machinery is essential for viral replication and packaging for release. Over millennia the biological arms race between the pathogen and the host has resulted in a range of measures on each side to combat each other. For example, there are very specific conditions normally required for RNA to be translated into proteins: each RNA should have a special cap, added by an enzyme, to mark it as ready for translation; it then needs to use over a dozen initiation factor proteins to get the small ribosomal unit (first part of the translation machinery) in position and to scan for the start sequence; only then is it able to assemble the full ribosome and proceed to protein synthesis.

It is thought there may be more than one billion species of virus with only a small proportion so far identified by science

Dr Kieft's group have shown how some viruses use RNA structures as a way around this process, and can instead attach directly to the ribosome and initiate translation without many of the normally necessary signals. Hepatitis C virus (HCV) has been shown to do this through what is known as an internal ribosome entry site (IRES). Structural elements of the RNA interact with the ribosome to correctly align the coding section independently of many protein factors. The team have then shown that even these structures can undergo conformational changes as part of the process to start elongation. This expands on our knowledge of the processes that underlie the central dogma of gene expression and shows that ribosome activity, fundamental to all living organisms, can be altered by bound RNA, even that originating from outside the cell.

Dr Kieft and his team are therefore bringing to light previously poorly understood processes and mechanisms. This gives invaluable insights – not just for novel therapeutics, but for our understanding of some of the most important cellular processes shared across all life.

Q&A

What makes RNA viruses such useful tools for studying cellular processes?
Viruses lack the basic machinery needed to make more copies of themselves, so they depend entirely on their ability to co-opt the machinery of infected cells. Many viruses have evolved ways to use their RNA to manipulate the processes that the cell has in place and that the virus requires to replicate. By understanding how the virus uses its RNA to manipulate specific cellular processes, we learn a lot about those processes and how they can be altered. Biology has had billions of years to evolve these RNA structures, so by exploring them we really are looking at highly tuned molecules that can teach us basic rules of how RNA structure relates to its activity.

How can this knowledge be used to create synthetic RNA with bioactive properties other than transient regulation of gene expression?
I think one of the most interesting things about structured viral RNAs is how the structure confers remarkable and unexpected properties and functions to the RNA. Understanding how some RNAs fold into complex and compact structures, what those structures look like, and how this drives function could potentially allow us to exploit these structures or design new ones. Part of my lab is working on applying the lessons that we learn from studying naturally-occurring viral RNAs to engineering new synthetic RNAs that do what we want.

Why is it important to expand our basic understanding of the biochemical processes inside cells?
Any hope of using science to improve human health or cure diseases requires that we fundamentally understand how biology works. We must always strive to answer the question: What are the basic molecular processes that govern biology (or the natural world in general!)? Basic science, motivated by pure curiosity and the desire to understand how things work at their most fundamental level, has always been (and will remain) the foundation upon which progress and innovation depends.

How might this help in the fight against devastating viruses such as Dengue Fever and HCV?
Understanding how these viruses interact with the cells they infect, and how this drives processes necessary for viral infection, is critical to find new targets for therapies. For example, if we find that a certain interaction or structure is playing a key role in a virus' ability to hijack the cellular machinery, then we can start to think about how we might block that interaction or disrupt that structure. If we lack this knowledge, then we are essentially flying blind in terms of finding new therapies.

Your latest work has found that certain RNA can be active in wildly different cell types, such as bacteria and animals, how is this possible?
Bacteria and animals are separated from one another by more than a billion years of evolution, but at their core, many of the processes and molecular machines remain very similar across all of life. Thus, in some cases a molecule that can alter a process in one species can also do so in very different species. Importantly, this means that scientists can use organisms like bacteria, yeast, fruit flies, nematode worms, zebrafish, and many others to explore the basic rules of biology and learn things that can be used to improve human health.

Basic science, motivated by pure curiosity, has always been (and will remain) the foundation upon which progress and innovation depends

Detail

RESEARCH OBJECTIVES

Dr Kieft's work focuses on viral RNA, aiming to understand as much about it as possible. His work is helping to elucidate not only the structure and function of RNA but also fundamental aspects of basic biological processes.

FUNDING

National Institutes of Health, Howard Hughes Medical Institute, American Cancer Society, Cancer League of Colorado, American Heart Association

BIO

Jeffrey Kieft graduated in 1990 from the US Military Academy at West Point, then served as an Army Officer in Germany. Upon leaving active duty, Dr Kieft earned his PhD from the University of California, Berkeley and did postdoctoral research at Yale University. In 2001 he was awarded the Roger Revelle/AAAS Fellowship in Global Stewardship, working as a member of the White House Office of Science and Technology Policy for one year before joining the faculty at the University of Colorado School of Medicine. He was an Early Career Scientist of the Howard Hughes Medical Institute and is currently a full professor with tenure.

CONTACT
Jeffrey Kieft
Dept. of BMG, Mail Stop 8101
12801 East 17th Avenue, Room L18-9110
Aurora, CO 80045

E: jeffrey.kieft@ucdenver.edu
T: +1 303 724 3257
W: http ://www.medschool.ucdenver.edu/KieftLab