Could you provide a brief overview of your work on influenza?

We look at host-microbe interactions by generating genetic snapshots of a virus population over the course of an infection and how it is affected by host immune responses. We capture this information by sequencing virus genomic RNA isolated from samples collected from the host respiratory tract. We map the mutations and define strains that persist, emerge and transmit across a population.

What was the inspiration behind the Influenza Genome Project?

This initiative was the first large-scale sequencing effort to characterise the whole genome, instead of just the two genes coding for the surface proteins of historic and circulating influenza virus strains. Using these sequences, we were able to map co-circulating strains within defined geographic areas across influenza epidemic seasons, and genetic mixing (called reassortment) that could explain vaccine failure. One of the project goals was to inform vaccine strain selection and identify potential for the emergence of pandemic strains.

In what capacity are you involved?

When the Project was initiated in 2004 at The Institute for Genomic Research (TIGR), I was Project Leader, developing the methodology and designing the first studies. When I moved to the University of Pittsburgh in 2006, the Project remained at TIGR (now the J Craig Venter Institute), and I became a close collaborator with the team that is currently led by Dr David Wentworth.

Can you explain how your research focuses on intra-host diversity, selection and evolution of influenza viruses?

The influenza A virus has a segmented genome where genetic information is encoded onto eight pieces of single-strand RNA. When we capture these RNA segments, we break the information into smaller pieces to decode their sequences. While we can reconstruct (or assemble) each segment into a consensus of what the genome of the dominant strain would look like, we cannot obtain individual genome information because the pieces cannot be unambiguously reassembled.

However, the information we do collect allows us to map the genetic diversity of the virus population by looking at all the short sequences that map across each gene over a sliding window. This information enables us to estimate the number of strains that are present and being transmitted, and to determine the proportion of genes that mutate over time and under certain conditions (such as immune pressure and drug selection).

To what extent could your work characterising intra-host influenza virus populations predict emergence?

We can highlight codons across each gene and the proportion of sequences that encode new amino acids. If these identified mutations are at known antigenic sites (in the haemagglutinin) or known drug resistance sites (on the neuraminidase or M2 proteins), we can estimate their frequency within a virus population and the potential for emergence of these mutations should host conditions be favourable.

Your project has observed the presence of oseltamivir-resistant pandemic A/H1N1 minor variants before drug therapy. Could you expand upon the importance of this finding?

It has been observed that drug resistance mutations in the influenza virus render the virus less fit, meaning that its replication and...
Influenza is recognised by the World Health Organization (WHO) as a serious public health problem. Seasonal epidemics cause severe illnesses and deaths for higher risk populations, and have a large economic impact through loss of workforce productivity and pressure on health services.

Each season, new strains of the RNA virus emerge with no cross-seasonal persistence. This makes influenza epidemics unpredictable and difficult to control. Research to further our understanding of this virus is critical to inform future prevention and response strategies against epidemics.

Professor Elodie Ghedin of the University of Pittsburgh School of Medicine in Pennsylvania, USA, and her team are investigating the intra-host diversity of influenza A viruses. This study is part of the Influenza Genome Project, an international collaborative effort for which a large part of the sequencing is based at the J Craig Venter Institute (JCVI) – formerly The Institute for Genomic Research (TIGR) – and led by Dr David Wentworth.

VIRUS GENOME MAP

The Influenza Genome Project was the first of its kind to characterise large collections of an acute RNA virus. Through this work, the team has already demonstrated the major role of genomic reassortment in influenza virus evolution, superseding outdated models of influenza evolutionary dynamics based on much more limited genetic data.

Influenza viruses have genomes made up of eight segments of RNA. Genomic reassortment occurs when two viruses infecting the same cell exchange segments of genetic material, thereby forming a novel combination. The new virus may have properties of both initial viruses, or completely new properties. This process is responsible for some of the major antigenic shifts of the influenza virus, such as the H1N1 swine flu outbreak. Ghedin’s research mapping the genetic sequences of whole influenza viruses has demonstrated this process and may identify new strains with the potential to cause a pandemic.

INTRA-HOST GENETIC DIVERSITY

When the H1N1 influenza A virus emerged in 2009, different variants were shown to be clustered by geographic region. Whilst the circulation of different strains of influenza is monitored and extensively investigated at a population level, the presence of multiple different virus strains within an infected individual had not previously been well characterised. Ghedin’s research has demonstrated extensive genetic diversity among influenza viruses within a given infected host.

Most studies on viral diversity within hosts investigate chronic infections, such as HIV or hepatitis C. In these infections, accumulation of errors acquired in replication and recombination promotes diversity of the viral population. Ghedin is investigating whether the same occurs for an acute infection like influenza, studying hosts to assess the genetic diversity of their viral populations. Novel methods are required to capture the true diversity within these individuals and examine how this changes within a host and during transmission between hosts.

NEW SEQUENCING PLATFORMS

The team uses high-throughput next-generation sequencing platforms – such as IlluminaHiSeq/MiSeq and PacBio RS – which, while of lower quality than previous methods, has allowed them to process more samples in parallel and provided individual genetic information on millions of molecules within a sample.

Ghedin reveals: “Initially, the sequencing pipeline for the project was based on the low-throughput but high-quality ‘Sanger’ technique”. Sanger sequencing generates a clean sequence of the dominant strain of the viral population within an infected host. Data from these new deep sequencing techniques

transmission potential is lower than for a wild-type virus. Thus, for a drug resistance mutation to persist, it has to have a selective advantage, i.e. pressure from the environment would force the virus to maintain this mutation in order to survive. What we observed in this study was that a small proportion of viruses carried a drug resistance mutation in the absence of drug pressure.

What are your plans for future research?

We are presently further exploring the concept of swarm transmission, i.e. that multiple virus strains are transmitted at the same time, and that in some cases defective viruses may be ‘hitching a ride’ with intact viruses, contributing to the genetic diversity found within the infected host. This may have an effect on host response to the infection.

We are also exploring how host immune status has a direct effect on evolution of the virus population over time. The question is whether vaccination pushes the evolution of the virus in specific directions and if this can be predicted.

Three current studies

Hong Kong Household study – sequence analysis of primary samples on index cases and their household contacts in a Hong Kong community during the first wave of the A/H1N1 pandemic

Social Mixing and Influenza Transmission Network Study in Schoolchildren (SMART) – analysis of influenza virus in the nasal swabs collected from children reporting influenza-like illness to determine whether the networks of transmission identified via contact information also track with the virus variants found in transmission pairs

Ferret Infection and Transmission studies – controlled experiments on naïve and preimmune ferrets, caged together, with influenza virus, to investigate the transmission and evolution of the pathogen

Ghedin’s research has demonstrated extensive genetic diversity among influenza viruses within a given infected host.
GHEDIN LAB: GENOMICS OF INFECTIOUS DISEASES

OBJECTIVES

• To develop and apply new genomic methods to study microbial pathogens, particularly the associations with their hosts in the context of human disease

• To identify the extent of intra- and inter-host microparasite (viruses and bacteria) diversity within the context of transmission and virulence, and parse the relationship between microbial ecology in the respiratory tract and disease progression

• To elucidate the molecular basis behind the adaptation of macroparasites, such as worms, to niches in their human hosts

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ELodie ghEdin is presently Associate Professor in the Department of Computational and Systems Biology at the University of Pittsburgh School of Medicine. In April, Ghedin will move to New York University to become Professor of Biology and Public Health, and a member of the NYU Center for Genomics and Systems Biology. She earned her BS and PhD degrees from McGill University in Canada. In 2011, she was named a MacArthur Fellow by the John D and Catherine T MacArthur Foundation.

THE FUTURE

Improving understanding of virus transmission and how this process affects viral diversity may be helpful in developing better influenza transmission models and reconstructing chains of transmission. This would be particularly important in developing future response strategies to manage influenza epidemics worldwide. Importantly, the next-generation sequencing technology used by Ghedin and her colleagues to analyse influenza sequence polymorphisms is providing a much richer genetic fingerprint from which to investigate viral transmission.

Ghedin’s work also has important implications for the field of vaccine development. The team aims to characterise the fitness distribution of all the variants found in a contact host and identify those that are transmitted, i.e. the extent of the population bottleneck. This knowledge should improve understanding of how viruses achieve transmission between humans, potentially providing new methods to interrupt this process and prevent epidemics. Whilst much more still needs to be learned before we can feel confidently prepared for any serious future epidemic, Ghedin’s research has already provided a platform from which greater knowledge of this ubiquitous disease can be gleaned.