Professor Mats Ljungman is leading the way in research into the regulation of gene expression. In this enlightening discussion, he talks about his work, his collaborations and his future plans.

How has your initial fascination in understanding the transcriptional dynamics influencing overall RNA content evolved?

The development of the Bru-Seq techniques, which began some 14 years ago, was sparked by an incomplete picture of the mechanisms of gene expression obtained by the assessment of total, steady-state RNA levels in cells using traditional approaches. We recognised that if we could label the newly made, nascent RNA and follow it, in time we would be able to estimate the rate of both synthesis and degradation of RNA.

In 2011, when we coupled our Bru pulse-chase labelling approaches with next-generation sequencing to form Bru-Seq and BruChase-Seq, the true power of these techniques became obvious to us. We have now expanded these approaches into four separate techniques each assessing a unique aspect of the regulation of gene expression.

What would you say separates your work from similar research in your field?

There have been a number of techniques that have been developed recently to assess nascent genome-wide RNA expression. These include global run-on and sequencing (GROSeq), nascent RNA sequencing (Nascent-Seq), native elongating transcript sequencing (NET-Seq), and metabolic labelling of nascent RNA by using microarrays or RNA-Seq. In many regards, these approaches work similarly to the Bru-Seq technique that we developed.

However, we have developed the additional approaches in the Bru-Seq suite of techniques where we incorporate a chase period to obtain the RNA population of defined ages (BruChase-Seq), irradiation of the cells with UV light prior to Bru-labelling to obtain information on active enhancer and promoter elements (BruUV-Seq) and finally the transient arrest and release of RNA polymerases to assess transcription elongation rates genome-wide (BruDRB-Seq).

Could you describe the Translational Oncology Program (TOP) that you are part of? What benefits has being part of such a network brought to your research?

TOP is a recently formed programme which has moved into a new state-of-the-art research building at the former Pfizer campus that is now part of the University of Michigan Medical School. The investigators in TOP focus on different aspects of cancer from basic to translational sciences. The TOP programme will expand in the next few years to include about 40 labs. The Bru-Seq technology platform will be used within TOP on a large scale to profile different cancer cell lines derived from primary human samples such as pancreatic cancer. We believe that this effort will create a very valuable resource for TOP and other researchers to understand mechanisms of gene regulation in different cancers.

Do you have plans for future research assays?

We are currently offering the Bru-Seq technology platform in collaborations with a number of individual research labs as well as the ENCODE consortium, which we are very excited to be a part of, to allow for a deeper understanding of mechanisms regulating gene expression. We are also interested in offering our services to pharmaceutical companies for testing how well their lead compounds are hitting their presumed molecular targets.

I believe that the use of the Bru-Seq techniques to explore individual signatures in human blood could be of tremendous value and, as sequencing costs are reduced, we think these techniques could be used routinely as a screening device for different human conditions.
Mapping the transcriptome

Through the identification and analysis of nascent RNA, scientists are furthering knowledge about the causes of aberrant gene expression in human disease. Research at the University of Michigan’s Cancer Center is leading the way with groundbreaking work into bromouridine labelling of nascent RNA.

RNA MOLECULES TRANSFER the genetic code from the nucleus to the ribosome. Recent developments in genome-wide methodologies have made it possible to identify and analyse all expressed RNAs simultaneously. By doing so for cancer cells, researchers can note the increased levels of specific RNAs and perhaps identify genes and proteins that might be associated with the cancer. Aberrant gene expression is also found in many inherited disorders and mental illnesses, and may contribute to the ageing process. To better understand and develop treatments for these conditions, there is a pressing need to track altered levels of RNAs, and to find out why they change.

Technologies such as RNA-Seq and ChIP-Seq are able to assess RNA levels and map epigenetic marks and transcription factor binding sites genome-wide. Yet, while these methods are able to generate signatures of steady-state levels of RNA and transcription factor binding sites, they are unable to identify whether a particular gene has altered transcription or RNA stability and therefore cannot pinpoint the cause of the altered levels of RNA.

In order to improve on these methods, researchers led by Mats Ljungman, Professor of Radiation Oncology at the University of Michigan’s Cancer Center, have developed groundbreaking sequencing-based methods. Their four complementary techniques – Bru-Seq, BruChase-Seq, BruUV-Seq and BruDRB-Seq – can obtain very detailed pictures of many aspects of transcription and its regulation not possible using other current methods. The techniques allow for the assessments of both the genome-wide rate of synthesis and degradation of RNA molecules. Furthermore, the techniques are used to map transcription start sites and enhancer elements as well as assess the transcription elongation rates of all expressed genes.

REPLACE AND REFINE

The group’s innovation lies in replacing uridine, one of the four building blocks of RNA, with bromouridine (Bru). Once labelled with Bru, this RNA can be specifically isolated from total RNA using specific anti-Bru antibodies and mapped using next-generation sequencing technology. A custom-designed analysis pipeline is then used to compare different aspects of gene expression between different samples.

Bru-Seq maps the nascent RNA and gives a genome-wide picture of ongoing transcription, which enables the team to determine transcription rates for all protein-coding and noncoding genes. BruChase-Seq ‘chases’ the Bru-labelled RNA in order to study RNA populations of defined ages. This technique allows for the assessments of splicing kinetics as well as the stability of mature RNA. BruUV-Seq introduces random transcription-blocking lesions by a pulse of ultraviolet (UV) irradiation prior to Bru labelling. This leads to enhanced signals at transcription start sites as well as at enhancer elements. By using BruDRB-Seq, the team is able to assess the transcription elongation rates across the genome. “Taken together, these four techniques obtain very comprehensive genome-wide signatures of a large number of important aspects of transcription,” Ljungman affirms.

CHASING THE DREAM

In a recent study, the researchers applied Bru-Seq and BruChase-Seq to human fibroblast cells and found that both synthesis and degradation rates for different RNAs varied wildly, suggesting that both of these processes are uniquely regulated for each transcript. When the cells were challenged with the pro-inflammatory tumour necrosis factor (TNF), many hundred genes rapidly changed their expression levels and the involvement of transcription and/or degradation in these alterations were recorded using Bru-Seq and BruChase-Seq. Some genes showed changes in transcription rates, transcript stability or both. The team identified 472 genes with induced transcription after an hour of TNF treatment while 204 genes showed a lower level of transcription during the same timeframe. Furthermore, 152 transcripts were significantly stabilised while 58 transcripts were destabilised following TNF treatment. “The results really emphasise how important the regulation of RNA stability is for cells when activating various cellular response pathways,” Ljungman adds.
INTELLIGENCE
DEVELOPMENT, VALIDATION AND APPLICATION OF THE BRU-SEQ TECHNOLOGY PLATFORM

OBJECTIVES
Developing innovative genome-wide methodologies and techniques to study the regulation of gene expression.

KEY COLLABORATORS
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PROFESSOR MATS LJUNGMAN received his PhD degree from Stockholm University in 1990 and performed postdoctoral studies at Stanford University from 1990-94. He joined the Department of Radiation Oncology at the University of Michigan in 1994 as Assistant Professor, was promoted to Associate Professor with tenure in 2001, and to full Professor in 2013.

Examples of data generated by Bru-Seq (blue trace) and BruChase-Seq (red trace) in human fibroblast cells. The figure on the left shows that the SMAD1 gene generates nascent RNA reads (blue) throughout the gene while 6-hour-old RNA (red) primarily shows reads for exons. On the right, the CALD1 gene shows that a few introns are being retained even after a 6-hour chase in these transcripts.

SUBTRACTING THE HAYSTACKS
The gene expression signatures generated by the Bru-Seq techniques are extremely rich and comprehensive. Bru-seq and BruChase-Seq provides synthesis and stability information on all RNAs adding up to more than 20,000 data points for each assay. In addition, using different periods of chase, the BruChase-Seq technique can generate splicing kinetics of potentially 200,000 introns. BruUV-Seq gives information on which promoter(s) is(are) being used for individual genes and also allows for the mapping of all active enhancer elements genome-wide. Finally, BruDRB provides estimations of the rate of transcription elongation for all larger expressed genes.

To analyse such large amounts of data, Dr Tom Wilson spearheaded the development of the computational tools for the Bru-Seq analysis pipeline. In addition to generating lists of gene expression features of a particular cell line, the scientists developed tools to rapidly compare samples. "This is where we think these techniques will be the most useful. It is like subtracting the haystacks, leaving only the needles," muses Ljungman.

CLARITY AND EFFICIENCY
The Bru-Seq technology platform has revealed a remarkably large number of influences on gene expression and putative regulatory elements, and has allowed them to be seen with more clarity and efficiency than ever before. The team is currently using the Bru-Seq techniques to explore a number of different biological questions, including investigating the effects of environmental agents such as ionising radiation, UV light and the heavy metal cadmium on the rates of synthesis and degradation of all transcripts in human cells. From there, they are also mapping enhancer elements that regulate the genes induced or repressed as well as monitoring effects of these agents on transcription elongation rates.

Alongside the work exploring environmental agents, the team is using the Bru-Seq platform to interrogate ageing, autism and cancer. Further research is also being undertaken into the role of epigenetic regulators in modulating transcription initiation and elongation as well as splicing and enhancer activation/repression.

A PERSONALISED FUTURE
Having applied Bru-Seq to human whole blood samples, the group is hopeful that they will obtain Bru-Seq signatures of individuals. These signatures could reveal various disease states and may provide information that could be used to inform the best treatment regimes for personalised medicine: "We are also interested in tracing exposure to environmental agents in gene expression signatures from blood of exposed individuals," Ljungman reveals.

As the cost of sequencing is falling, it may be feasible to use the Bru-Seq techniques in the doctor’s office to obtain individual signatures from a blood test. Specific changes in these signatures over time may indicate the onset of, or the predisposition to, a certain disease state prior to the appearance of other symptoms and may thus provide opportunities to start early preventative treatments, as Ljungman concludes: “We are very excited about the prospect that the Bru-Seq technology platform may one day have clinical utility”.

The Bru-Seq technology team. From the left: Karan Bedi, Jayendra Prasad, Mats Ljungman, Michelle Paulsen, Hanna Ljungman, Ben Biewen, Leo Lima, Jerry Oommen, and Artur Veloso. Not shown: Tom Wilson, Brian Magnuson, Killeen Kirkconnell, Brian Lu, Emily Ljungman.