Background

Researchers often see RNA as the temporary molecule between DNA and proteins. While this is part of the story, RNA also regulates various biochemical processes inside and outside of cells. When RNA exists outside the cell, we call it extracellular RNA (exRNA).

We believe exRNA in blood, for example, can help diagnose diseases, such as cancer or Alzheimer's disease. To explore their potential, we first need to take a step back and question how we study exRNA: *What tools can we use?* and *How do these tools work?* Then, we can employ this knowledge and uncover the intricacies of exRNA.

Research aims

The aim of this thesis is to deepen the understanding of exRNA complexity and facilitate biologically relevant discoveries in this field. I tried to do so by first highlighting critical exRNA sequencing pitfalls (Paper 1 and Paper 3), developing research methods (Paper 2), and then using these to make discoveries on the biology of exRNA (Study 4).

Results

At the start of my Ph.D., we noticed that the results of an influential exRNA-seq paper suffered from DNA contamination. Thus, I wrote **Paper 1** in response to this article. This letter now also stands as a plea for thorough DNA contamination control.

In **Paper 2**, Prof. Everaert and I developed a method to very specifically exclude highly abundant transcripts from the RNA-seq library preperation. We showed this novel approach works for small, 3'-end, single-cell, and long-read RNA-seq.

Most RNA-seq protocols include a step to turn RNA into complementary DNA. This step is called 'reverse transcription'. However, this step introduces biases and artifacts because some RNA is easier to turn into DNA than others. To bring this to light, I wrote **Paper 3**, a review on this topic.

About two years ago, I started developing the first method for sequencing exRNA using long-read sequencing. I have since used this approach to show that intact messenger RNA molecules are present in human blood plasma, urine, and in different subcompartments of these biofluids. Although this project is not published, I included it in my thesis as **Study 4**.

Conclusion

The fruition of my thesis shows that a combination of critical evaluation and protocol optimization can lead to highly relevant and novel discoveries.

If you remember 5 things...

1. Your cells use DNA as a template for RNA, which can then enter your blood, urine, tears, ...
2. DNA can contaminate RNA research.
3. The most interesting RNA is often rare and overshadowed by abundant RNA.
4. We introduce biases and artifacts when we turn RNA into DNA for analysis.
5. Intact RNA exists in human biofluids.

Research included in my thesis

Verwilt, J, Trypsteen, W, Van Paemel, R, De Preter, K, Giraldez, M D, Mestdagh, P, & Vandesompele, J (2020). When DNA gets in the way: A cautionary note for DNA contamination in extracellular RNA-seq studies. Proceedings of the National Academy of Sciences, 117(32), 18934-18936.

*Verwilt, J**, Everaert, C*, Verniers, K, Vandamme, N, Marcos Rubio, A, Vandesompele, J**, & Mestdagh, P** (2023). Blocking abundant RNA transcripts by high-affinity oligonucleotides during transcriptome library preparation. Biological Procedures Online, 25(1), 1-19.

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Verwilt, J, Verniers, K, De Geyter, S, Roelandt, S, Pinheiro, C, Hendrix, A, Mestdagh, P** & Vandesompele, J** (2023). Quantification of Intact Messenger RNA in Human Blood Plasma and Urine, and Their Purified Fractions. In preparation.

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Research not included in my thesis

Verwilt et al. Scientific Reports, 12(1), (2020)
Evans et al. Clinical chemistry, 68(1), (2022)
Verwilt et al. bioRxiv, 2020-09, (2020)
ExRNAQC Consortium bioRxiv 2021.05 (2022)

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Scan to read my thesis!

Personal note

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Advancing Extracellular RNA Research

Through Method Scrutiny, Optimization, and mRNA Integrity Profiling

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