THE MESSENGER

Newsletter No. 15

July 2021 — National Center of Competence in Research, RNA & Disease

NCCR
RNA & Disease

National Center of Competence in Research
The role of RNA in disease mechanisms

Dear colleagues

Our lives have been substantially influenced by the SARS-CoV-2 pandemic for more than a year now. We got used to keep distance from each other, spent countless hours on Zoom, canceled travel plans and are longing for eventual in-person meetings to become possible again. While our research was certainly also negatively affected by temporary lab shutdowns and reduced possibilities for exchanging thoughts and ideas with each other, the pandemic has also provided unexpected advantages and opportunities for our NCCR RNA & Disease: *Suddenly, the broad public became interested in learning about RNA viruses and how to fight them with mRNA vaccines, whereas previously one of our biggest challenges in public outreach was to convey what "RNA" is and why it is important to investigate. In that respect, the timing for developing the* Molecool – Cosmos RNA *website turned out to be perfect. If you haven't done so yet, check it out. That the cumulative expertise on RNA represented in our network was clearly sought-after during the past year is also exemplified by the many interviews in various media given by our NCCR members.*

As many of you know, our colleague Steve Pascolo is one of the pioneers in developing mRNA vaccines and a co-founder of CureVac, and I am sure that the interview with him, which is the centerpiece of this Newsletter, will provide a highly interesting read both for the RNA geeks among you as well as for RNA-curious lay persons.

Oliver Mühlemann
Director NCCR RNA & Disease

Interview Steve Pascolo

"You mean I am a liar or what?"

Interview: Dominik Theler

In this interview Steve Pascolo tells us about the history and perspective of mRNA therapeutics and his career.

A bit over 60 years ago, Yuri Gagarin was the first human in space. As far as you know, you were the first human to be injected synthetic mRNA coding for luciferase for experimental purposes: Do you see yourself as the Yuri Gagarin of mRNA therapies?
Not at all. He had to be much more courageous than me. RNA is quickly degraded, and similar experiments were conducted before in mice, so I knew there would be zero risk. The question was if we could detect luciferase activity. Besides, I was responsible of the pharmaceutic production of synthetic mRNA that I put in place in CureVac so I knew that the product is safe!

Nowadays, millions of humans are vaccinated with mRNA against SARS-CoV-2, but at the beginning there was a lot of skepticism as you indicate with the title of a recent review "Synthetic Messenger RNA-Based Vaccines: from Scorn to Hype". Why that?
Because of a prejudice in the scientific and medical communities that had the textbook knowledge in their mind that mRNA is fragile. When I was showing the data to scientists, many of them were telling me *"Ah no, it cannot work."* and I replied, *"You mean I am a liar or what?"* However, investors did not care about this prejudice when we could show them the data that mRNA-based vaccines work. A lot of people ignored the potential of mRNA vaccines even until as recent as 2019.

Did this scorn cause you a lot of frustration?
No, on the contrary. In a way, it was fun to fight. I knew that mRNAs will be one day used as drugs and will attract a lot of interest. From 1998 until like 2006, we as CureVac were alone, we were a small team and you are never so strong than when you are facing opposition. It creates a team spirit of taking up the challenge demonstrating them that it works. It was not frustration, on the contrary, it caused a type of energy.

The only thing that frustrated me came later relating to our early publications describing the luciferase injections into me and the first clinical study of mRNA vaccines (done in melanoma patients), which were published in 2007 and 2008, respectively. Both we only could publish in low impact factor journals. At the time, I did not care too much and was just happy that the results got published and the community had access to them. I got frustrated later, for example when our early work was not cited, although I know from personal communication that it inspired other vaccine developers in their experiments.

When did you start to research mRNA vaccines?
For my postdoc, I went to the lab of Hans-Georg Rammensee in Tübingen. I started to compare peptide, protein and DNA based vaccines against cancer to see which one works best. In the Rammensee lab, I met Ingmar Hoerr, who was about to finish his PhD studies on mRNA vaccines. I went to Ingmar and told him that I would like to include mRNA vaccines in my comparison. Then, I tested the mRNA vaccine and it worked. However, it was less effective than the DNA one, but I was intrigued by the safety and production advantages it could provide.

Interview Steve Pascolo

"You need to have this big vision that you will not build a house but a building."

Ingmar was not too much into science and wanted to do business. So, after his PhD he went to do an MBA but he was still then and when coming to the lab. During his visits, I would tell him about the results I had with mRNA vaccines. As part of his MBA, he had to write a business plan and he wrote it on an mRNA company. He then proposed to create CureVac. Ingmar and I were a good team, because I was really into science and he really into business while still understanding the science well.

What does it take to be a good entrepreneur?

Image Credit: USZ/Christoph Stulz

Steve Pascolo

Biography

Steve Pascolo studied Biology & Biochemistry at the Ecole Normale Supérieure in Paris, France and then conducted his PhD studies as well in Paris at the Institute Pasteur. In 1998, he moved to Tübingen, Germany for his postdoc in the lab of Hans-Georg Rammensee. In 2000, he co-founded CureVac and served as its Chief Scientific Officer until 2006. Afterwards he moved to the University Hospital Zurich, where he is currently a private lecturer and group leader in the Dermatology Department and head of the mRNA platform of the University of Zurich's University Research Priority Program Cancer. In 2008, he founded Miescher Pharma, which provides consulting services and licenses a patented invention on Protamine mRNA. In 2017, he became an associate member of the NCCR RNA & Disease.

UZH URPP Cancer mRNA Platform

I think you need to have a long-term big vision and self-confidence. You need to have this big vision that you will not build a house but a building. I am more the type that would build a studio, while Ingmar is more the type that wants to build a skyscraper with 150 floors.

You left CureVac in 2006 to go to the University Hospital Zurich (USZ) with a postdoc contract, have you ever regretted this decision?
No, not at all. I would have regretted leaving if later CureVac had failed as a company. Then I would have thought *"Oops, maybe if i had stayed, the company would still exist."* When I left, I had the feeling that CureVac was on track and somebody else can take over as CSO. At that time, I did not like my job anymore because I spent all the time in the office and not doing any lab work. I was waking up and thinking *"Damn, I have to go to work."* and that was a no-go. So, I decided to go back to lab work, which is a lot of fun for me.

"I was waking up and thinking 'Damn, I have to go to work'."

In hindsight, I underestimated how much more data the regulatory authorities would still ask for and that the clinical studies still needed improvement. When I left, CureVac was the only company and only later competitors like BioNtech and Moderna emerged. In the end, I think it was not wrong to leave CureVac. The company did well without me, and I did more or less well without the company except the two years from 2010 to 2012 when I lost my job at the USZ.

Can you compare BioNtech, CureVac and Moderna?
The stories of BioNtech, CureVac and Moderna are quite different: At CureVac we were a team of basic scientists, who wanted to further develop and optimize mRNA vaccines bringing them into the clinic. The initial drive behind CureVac was scientific curiosity.

BioNtech had pure clinical roots: Özlem Türeci and Ugur Sahin were treating cancer patients. They founded two companies to help their patients because when they presented their ideas to pharmaceutical companies, the companies did not want to pursue these ideas.

In 2010, American investors felt that mRNA could do a lot, so they gave the capital and they hired people for developing the therapies. The Moderna history is business, which is good as well. So, CureVac was scientific, BioNtech medical and Moderna I would say was business. I think it is good that these three companies have different energies driving their developments.

You state in interviews that you are not affiliated or have stocks neither in BioNtech, CureVac or Moderna. Still, you are listed as an inventor on 26 patents. Do you benefit from these financially?
Regarding the patents relating to CureVac, no. I gave up the rights of those to the company. The only patent I profit from is one that I filed as an USZ employee in 2008 on protamine-RNA nanoparticles, which was licensed and which I get a little bit of royalties from. I filed two more patents while being at the USZ. One on an optimized 5'UTR of the mRNA, which became obsolete after other researchers developed even better 5' UTRs. In 2018, I filed a patent on a new type of chemically synthesized mRNA. If it gets approved and can be licensed, I could get royalties from. So, from the mRNA pioneers, I am probably the only one who will not get personally rich.

Do you expect that you will more easily acquire research funding?
Yes, that would be a great thing. The problem I have with my lab is financial sustainability and stability. I envisage at the University of Zurich (UZH) an mRNA department with three to four fixed positions so that we have a core of people whose salary is not dependent on successfully acquiring every two to three years the next grant. This would not only lead to stability but also institutional visibility, which is a goal I have so far not achieved.

If we get a few permanent positions and a real lab space for mRNA research, then we could plan projects long term. This would lead to patentable inventions, which could bring revenues for the University. This type of research is hard to do with the current unstable financial situation we have. With more stability we could really be part of the game and foster mRNA research in Zurich and Switzerland.

"If Switzerland wants to have a BioNtech, CureVac or Moderna in the future, it is possible."

Interview Steve Pascolo

In April 2021, the only support I have from the UZH is from the University Research Priority Program Cancer (70'000 CHF per year till 2022), which started in 2016 and a small grant (28'200 CHF) for mRNA research in 2021. But there is no strong support from the USZ or UZH for mRNA research. I got an EU grant and an SNSF grant in 2020 on mRNA, which never happened in the past, but we now need to shift to second gear. We need a bigger lab and we need a GMP production facility for mRNA.

If Switzerland wants to have a BioNtech, CureVac or Moderna in the future, it is possible because there are big possibilities of using mRNA and great scientists in Switzerland but there needs to be support for this. Politicians still need to understand the big potential of mRNA and decide if Switzerland wants to be part of it. I talk to journalists so that the public and the politicians understand the mRNA technology in the vaccines but also beyond this, the great potential of the mRNA technologies in medicine.

Also, I suggested that the army should have an mRNA platform if they want to protect the Swiss population. Maybe the combat jets, which cost billions, are important but with a few millions invested into an mRNA platform including production, the Swiss people could be protected against emerging infectious diseases.

We need to see big and be part of the mRNA development in the world. This depends on political decisions to really go quick. And not only saying "We see the potential it has" and "Switzerland should be part of the development of mRNA therapies in the future".

You developed in your lab also an mRNA vaccine against SARS-CoV-2 and injected it into mice. Did it work as far as you could tell? And if yes, were you tempted a year ago to vaccinate yourself with it?
Yes, the vaccine according to the animal experiments worked. And no, I was not tempted to vaccinate myself with it because the liposome we have is designed for vaccinating cancer patients by intravenous injection yielding mostly type 1 interferon and T cell response. It is not suitable if you want to have high levels of antibody titers. We lacked a good mRNA formulation for high level antibody production, but we are now working on that with the support by the Swiss National Science Foundation. Myself, I have recently received the second BioNtech shot, so in total I have received five injections of mRNA so far during my life.

What are the current research directions to optimize the mRNA technology?
One is to get the technology working with less and less mRNA required. The less you need to produce, the faster you can get the mRNA to billions of persons in need. Also, the distribution would be facilitated if formulations were found, which allow to store the mRNA formulations for a long time at room temperature.

A lot of the pre-pandemic mRNA therapy research was focused on cancer therapies: Will we see a breakthrough in this area soon?
I hope so. BioNtech together with Genentech is currently conducting a Phase 2 trial with mRNA vaccines for treating melanoma. They combine the mRNA vaccine with an immune checkpoint inhibitor. So far, using a vaccine alone to treat a cancer has been shown to be not sufficient whether you use vaccines based on mRNA, peptides, proteins, viruses or DNA. So, it was not the mRNA itself that was the problem why the first mRNA vaccines against cancer did not work.

Nearly every vaccine type has been tested against cancer and none of them have been successful. They do induce an immune response but the immune response over time does not control the tumor. We need combinations and we will see how it evolves but the vaccines against cancer mutations developed by BioNtech are very potent because they on one hand give type I interferon response and on the other hand T cells directed against the tumor mutations. I hope this development will lead to approved mRNA vaccines against cancer. I think once the door is open there will be a lot of followers.

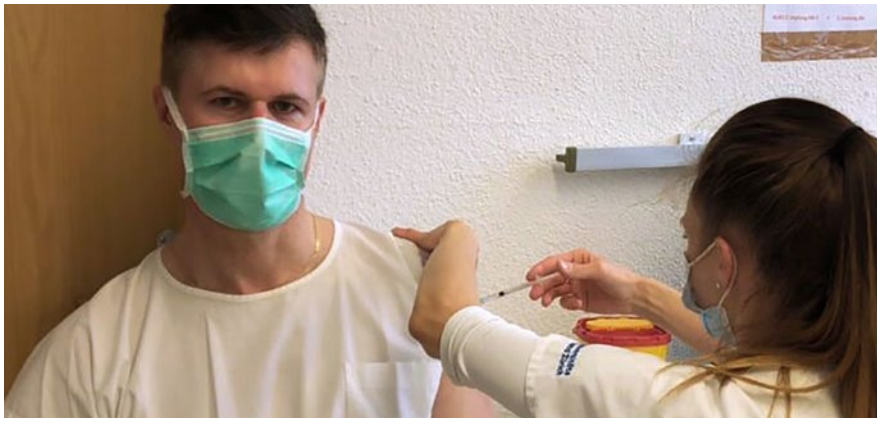
Also, mRNA has great potential for chimeric antigen receptor T (CAR-T) cell therapies. The current CAR-T cell therapies are based on gene therapy with DNA. When you use mRNA, you have the advantage that it is transient, so you do not have the long-term risk of cells proliferating causing a cytokine storm or encephalitis as it has been seen in some patients.

When the tumor starts to escape by mutating and you want to redose the patient with DNA-modified T cells carrying a different CAR, you might have accumulation of the previously injected CAR-T cells increasing the risks of leukemia, cytokine storms and these types of adverse reactions. This concern does not exist with mRNA modified T-cells.

You can make with mRNA CAR-T cells that would be active for 2 or 3 days and dose the patient every week in order to get rid of the tumor. When the tumor becomes resistant to this CAR then you can continue treating with an mRNA coding for another CAR and continue the treatment without the risk of having accumulation of previously injected CAR-T cells.

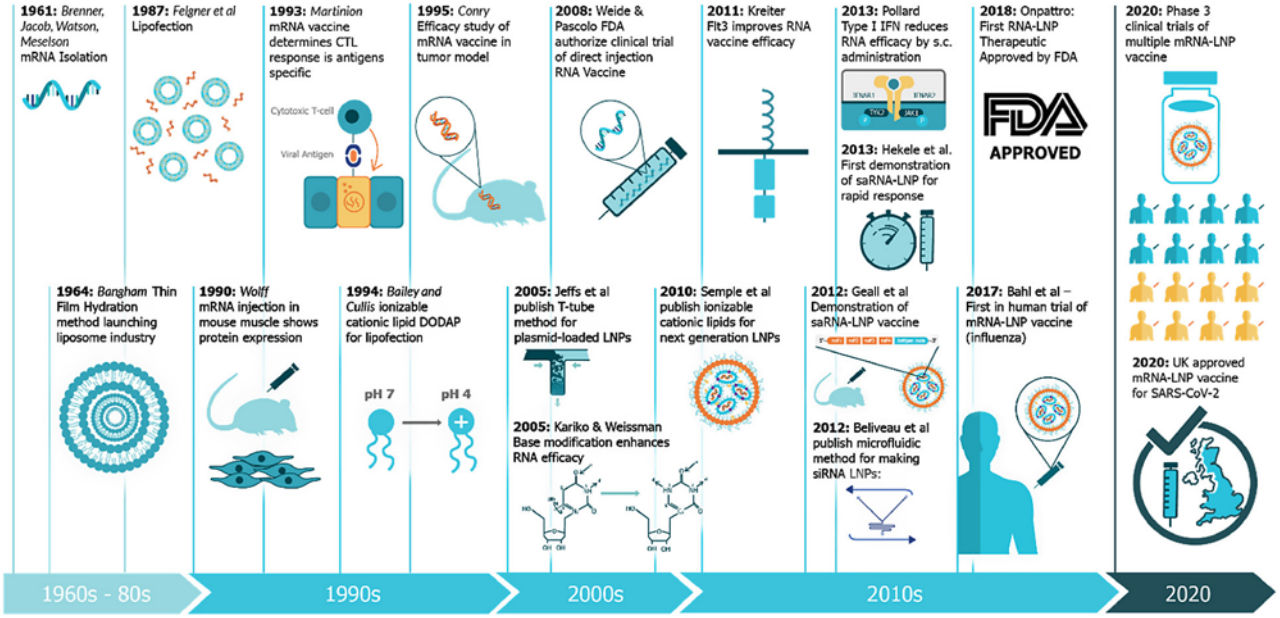
What other therapeutic areas do you see for mRNA-based therapies besides cancer and infectious diseases?
It is very broad. For example, mRNA is great for the area of regenerative medicine. If you want to regenerate damaged tissue, you want to do this temporarily. For this mRNA is perfect. On top of that, mRNA has great potential for personalized medicine and treating rare genetic diseases.

Back to the start of mRNA therapeutics research: In the media, Katalin Karikó and Drew Weissman are frequently mentioned as the scientists having made groundbreaking discoveries regarding mRNA vaccines. They pioneered the use of modified mRNA for therapy but not the use of mRNA as a vaccine?
Exactly, everybody talks about them, but this story is not complete like that. They disclosed in 2005 that modified mRNA is not immunostimulating, allowing it to be used for therapies – the exact opposite of vaccines: the injected mRNA does not trigger inflammation and consequently there is no immune response against the encoded protein. Kati has done a lot of great work on mRNA ther-



Steve Pascolo receiving his second BioNtech vaccine shot making it the fifth mRNA injection for him. Source: LinkedIn post by Steve Pascolo.

Interview Steve Pascolo



A timeline of innovations that have contributed to the development of self-amplifying RNA vaccines and associated technologies. Figure from Blakney et al. (2021) An Update on Self-Amplifying mRNA Vaccine Development Vaccines 9(2), 27. Published under a CC BY 4.0 license.

apies, but to my knowledge, you do not find in the literature an article on mRNA vaccines with her as first or last author. Drew worked on mRNA vaccines and published in 2017 a Nature paper as last author on the usage of modified mRNA for vaccination. This came as a surprise, because modified mRNA was not supposed to cause inflammation, which is required for vaccination. So far, it is not known whether modified or non-modified mRNAs are better for vaccines. The anti-cancer mRNA vaccines in development by BioNTech are all non-modified. I think this media attention comes from the fact that the communication of the Americans including their universities is unbeatable and we do not have this culture here.

I am always telling people that it started in 1990 by Wolff and colleagues showing for the first time that synthetic mRNA can be used *in vivo* to induce protein expression by injecting naked mRNA intramuscularly in mice. Regarding the use of mRNA for vaccination, the breakthrough was in 1993 when Martinon and colleagues published that mRNA produced *in vitro* and put into liposomes can be used as a vaccine.

However, this publication had also a bad side because the authors did not believe themselves in the technology to the extent that they filed a patent but then abandoned it. So, this led to using mRNA in liposomes as being in the public domain. When we founded CureVac we did not have the means to develop new patentable liposome formulations, so we started using protamines that we could get a patent on. You can jeopardize the development of a technology by not patenting it and just putting it into the public domain.

In the last couple of months, you were in my impression omnipresent in the media in France and Switzerland. Why are you doing this?

I see it as my role to inform the public in a neutral way. It is an advantage that I do not have shares from any of these companies and I can say freely what I think. We are now in a new dimension because of the problems with the AstraZeneca vector vaccine, and people want the mRNA vaccines. However, we should not forget that in December 2020, a lot of people were skeptical about mRNA vaccines. Then it was my role to explain to the general public what mRNA is and that it is degraded quickly, efficient and safe as vaccine, so that then everybody can decide themselves whether they want to get vaccinated with mRNA or not.

Also, I had to go to the media to counteract statements made by pseudo experts that were saying *"Oh no, the mRNA vaccine: we do not know what is inside and we should wait"*. That is horrifying to me because people were dying in vain. We had the vaccine and then "experts" come on TV seeding doubts in the public. There were some people on the radio saying that because of the skepticism seeded by the pseudo experts, they did not want their elderly father to be vaccinated, who could have been vaccinated with mRNA as early as January 2021. In March, he died from Covid-19. It was very important at that time to explain what mRNA is and stress that the whole development of mRNA vaccines and therapies is based on safety advantages of this format.

What were the reactions you got from the public after your media appearances?

Most reactions were positive and people wrote to thank me that they now understood what mRNA vaccines are, and that they will get vaccinated. This type of reactions showed to me that the time spent talking to and on the media was worth it. There was also a minority of persons accusing me that I do this media work for money, but I did not get any financial benefit from media or from mRNA vaccines against Covid-19.

> *"To be innovative and not redo what other people did"*

With your experience in academia and starting a company, what advice would you give to young researchers?

To be innovative and not redo what other people did. Also, to not stick too much to the textbook knowledge, of which not all is written in stone. As it was the case of mRNA, which was described as too fragile to be used for therapeutic applications. Dare to go a different route than everybody else, which is not without risk.

That said, I am not rich, I was two years without a job, I am not a professor and have a 20 square meter lab, so I am not sure if I am the right person to give career advice, since a lot of people my age are doing much better than me. Still, even if the price to pay was a slower career and lower salary, I am still happy with the career path I took.

Interview conducted on April 26, 2021.

Research Highlights

Splicing inhibition by a single methyl group

Dominik Theler

In humans, the majority of N^6-methyladenosine (m^6A) is deposited by the heterodimer METTL3/METTL14. However, a subset of marks is placed by the writer enzyme METTL16. The Pillai lab previously gained insights into the structure and function of this writer in mammalian organisms (Mendel, Chen et al., 2018).

To further study this writer, they turned to the METTL16 homolog in *C. elegans* called METT-10. *C. elegans* is an interesting model to study, as in contrast to mammals, it lacks a METTL3/METTL14 homologue. For the worm research, the Pillai lab joined forces with a lab headed by another Schümperli group alumnus Florian Steiner (both at the University of Geneva). Little did they know at the beginning, that this research would lead them into the RNA splicing field, discovering a novel mechanism for its regulation.

As a start, they mapped the m^6A transcriptome of *C. elegans*. Despite the similar amount of m^6A in *C. elegans* and mouse, they identified only 176 m^6A peaks in worms, while a similar analysis in mouse identified over 20 000 peaks. Upon knockout of the m^6A methylase METT-10, they observed that the m^6A methylation levels went down for several transcripts, including sites located in the transcripts of U6 snRNA and *sams-3, -4 and -5*, which are SAM synthetase transcripts. SAM is the most important methyl donor for methylation reactions in the cell, including m^6A deposition. The identification of these sites mimics the situation in mammals, in which U6 snRNA and as the SAM synthetase *MAT2A* are targets of METTL16.

However, the location of the methylation sites in the SAM synthetase transcripts differs in mice and worms. While the mouse *Mat2a* has six methylation sites in the 3′ UTR, the transcripts of *C. elegans sams-3, -4 and 5* are methylated at a single site, which is the 3′ splice site AG of the intron 2 in the *sams* pre-mRNA transcripts. Methylation of the 3′ splice site leads to splicing inhibition of the corresponding intron and to reduced levels of the SAM synthetase. Through further experiments, they could show that the m^6A mark at the 3′ splice site prevents binding of the essential splicing factor U2AF35, inhibiting splicing.

The researchers could further show that the methylation of 3′ splice sites only occurs under high nutrient conditions. Under low nutrient conditions, the methylation mark is absent and splicing produces mature transcripts. *"The connection to nutrient levels seems obvious, but we discovered it initially by chance"* says Ramesh Pillai, last author of the study. When performing the final round of biological replicates, the worms were grown accidentally on low-nutrient plates and the methylation at the 3′ splice sites disappeared. *"At first, this came as a shock because we thought our initial experiments and model were wrong. But then, we went with Kamila Delaney (PhD student in the Steiner lab) through the lab journal entries, and we noticed the usage of different plates."* recalls Mateusz Mendel, first author of the paper and PhD student in the Pillai lab.

In conclusion, they could show that both *C. elegans* and mammals use a mechanism depending on the homologous m^6A methylases METT-10 and METTL16, respectively, targeting SAM synthetase transcripts to regulate SAM levels in response to nutrition. However, the underlying regulation mechanism in the two systems is different. In response to high SAM levels, worms downregulate SAM synthetase levels through METT-10-mediated 3′ splice sites methylation, leading to splicing inhibition. In mammals, it was previously shown, that METTL16 promotes the splicing of the Mat2a transcript when SAM levels are low. However, this is not through its catalytic activity, but through vertebrate-specific regions located in its C-terminal half. When SAM levels are high, mammalian METTL16 methylates its *Mat2a* binding sites and dissociates from the transcript, leading to loss of splicing promotion. This methylation sites further fine-tune *Mat2a* levels through recruitment of an m^6A reader YTHDC1 to promote degradation of the *Mat2a* transcript.

The big question then was if methylation of 3′ splice sites can control splicing in mammals. Through experiments with splicing reporter constructs *in vivo* using HeLa cells, and *in vitro* with HeLa S3 extracts, the researchers could demonstrate that m^6A methylation of the 3′ splice site can inhibit the human splicing machinery. Moving beyond the reporter constructs, computational analysis revealed the existence of around 1000 splice sites in the mouse transcriptome that could potentially be regulated by METTL16. In an *in vitro* methylation assay, the majority of the top ten most promising sites could be methylated by METTL16. Moreover, through sequencing the transcriptome of *Mettl16* knock out mouse embryos, they identified two splice sites, whose use is upregulated in the absence of METTL16. These results do hint, but not finally prove, that these sites are regulated directly by METTL16. The findings of the Pillai and Steiner labs have just been published in Cell (Mendel et al., 2021). Further research could help to clarify if 3′ splice site methylation by METTL16 is a splicing regulatory mechanism also conserved in mammals.

Cover image by Marzia Munafò kindly provided by the authors.

Mendel et al. (2021) Cell 184(12), 3125-3142.e25 (Open Access)

Mendel, Chen et al. Molecular Cell 71(6), 986-1000.E11 (Open Access)

Research Highlights

Spotting the Unexpected: Translation in Stress Granules

Veronika Herzog

When cells are exposed to diverse stress stimuli (e.g., viral infection, heat or oxidative stress), they activate the *integrated stress response (ISR)* pathway to promote cellular recovery. This pathway results in a decrease of global protein synthesis, an induction of selected genes and the assembly of cytoplasmic membrane-less organelles termed stress granules (SGs). For a long time, it was widely believed that SGs promote the translational repression of RNAs and that they contain exclusively translationally-repressed RNAs. A recent single-molecule imaging study from Jeffrey Chao's lab at the FMI Basel now challenges this notion *(Mateju et al., Cell, 2020)*.

To characterize the relationship between mRNA localization and translation during stress, Mateju and colleagues aimed to simultaneously image individual mRNA molecules and the associated nascent polypeptide chains at defined sub-cellular localizations. To do so, they engineered a reporter construct containing the 5' UTR of ATF4 (a gene that is induced upon stress conditions), followed by a coding sequence encoding a SunTag array and MS2 stem-loops in the 3' UTR. Upon integration and expression of this reporter construct in HeLa cells, the individual reporter mRNAs and the nascent peptide chains were visualized in single-molecule imaging experiments via the MS2 stem-loops and the SunTag, respectively. Combining these experiments with live-cell imaging of SGs under oxidative stress conditions enabled the analysis of translation activity of the reporter mRNAs both in the cytoplasm and SGs in stressed cells.

As expected, the authors observed many non-translated mRNAs (i.e., mRNAs devoid of a SunTag signal) in SGs. But surprisingly, ATF4-SunTag mRNAs undergoing translation are also found in SGs. In fact, about 30% of ATF4-SunTag mRNAs localized to SGs are translated, suggesting that SG-associated translation is not a rare event for this type of transcript. *"I was very surprised to see translation activity in SGs. Initially, we were skeptical that SG-localized mRNAs could be translated efficiently, but with further experiments and quantitative analysis we became more convinced."*, says Daniel Mateju, first author of the study.

To exclude the possibility that the mRNAs with nascent polypeptides in SGs are aberrant or stalled translation complexes, Mateju and colleagues sought to further characterize SG-associated translation. First, by acquiring longer movies, they identified SG-localized mRNAs initially devoid of SunTag signal but acquired SunTag signal over time, indicating that translation initiation can take place in SGs. Second, the authors quantified elongation rates and found that the elongation rates of SG-localized and cytoplasmic mRNAs are comparable. And third, by assessing the kinetics of SunTag signal accumulation of SG-localized transcripts in time-lapse movies, they concluded that ribosomes are able to terminate translation in SGs. Thus, mRNAs can undergo the entire translation cycle (initiation, elongation and termination) in SGs and SG-associated translation resembles translation in the cytosol.

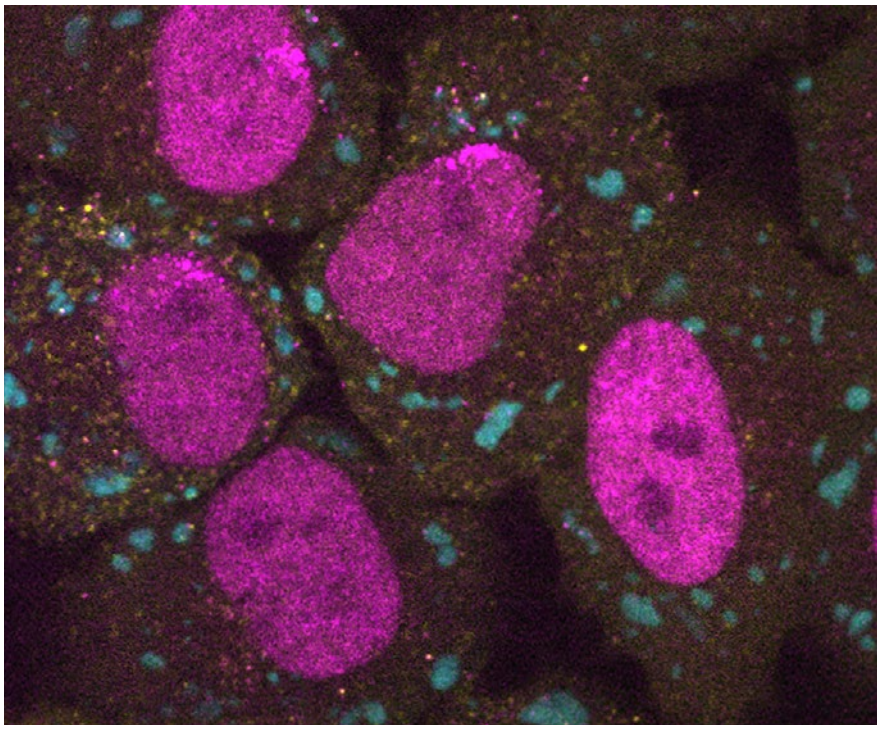


Image of cells containing stress granules (cyan) and ATF4-SunTag mRNAs (magenta foci), some of which undergo translation (yellow foci). Picture kindly provided by Jeffrey Chao.

The authors further obtained evidence that SG-associated translation is not limited to stress response mRNAs such as ATF4. When the 5'UTR was replaced with one containing a 5'TOP motif (a *cis*-acting motif found in genes that are repressed during stress), this reporter mRNA was strongly translationally repressed. Only a few 5'TOP reporter mRNAs were found to be translating during stress and those were found not only in the cytosol but also in SGs. Taken together, these results show that mRNAs localized to SGs can undergo translation and suggest that the SG environment *per se* does not induce translation repression.

The recent technological developments in single-molecule analysis have revolutionized the way the function of membrane-less organelles or biomolecular condensates can be dissected and open new avenues for future research. Jeffrey Chao, corresponding author of the study, is excited about this development: *"For a long time, we could only observe stress granules and look at the juxtaposition of stress granules and P-bodies. Now we can start testing the models that have been proposed and find out which ones are correct and which need to be re-evaluated."*

Publication:

Mateju et al. (2020) Cell, 183(7), 1801-1812.e13

Research Highlights

SARS-CoV-2 depends on the ribosome missing a step

Dominik Theler

Many viruses depend on ribosomal frameshifting for the expression of their proteome and replication. Such viruses include HIV, Influenza A as well as all known Coronaviruses. Coronaviruses require a -1 frameshift so that its open reading frame (ORF) 1b in addition to the ORF1a can be translated. ORF1b encodes for the RNA-dependent RNA polymerase and additional proteins crucial for viral replication. Although frameshifting is critically important for the replication of medically relevant viruses, so far, no detailed molecular image of the process could be obtained.

To address this question, the Atkins (University of Cork, Ireland) and the Ban labs (ETH Zurich) teamed up and were later joined by the Bode (ETH Zurich), Gatfield (University of Lausanne) and Thiel (University of Bern) groups. Initially, the researchers established the biochemistry to trap the ribosome in order to investigate a cellular frameshifting event. *"When the Covid-19 pandemic broke out, this previous research allowed us to rapidly switch to study the frameshifting site of the SARS-CoV-2 virus."* says Alain Scaiola, co-first author of the study published in Science and PhD student in the Ban lab.

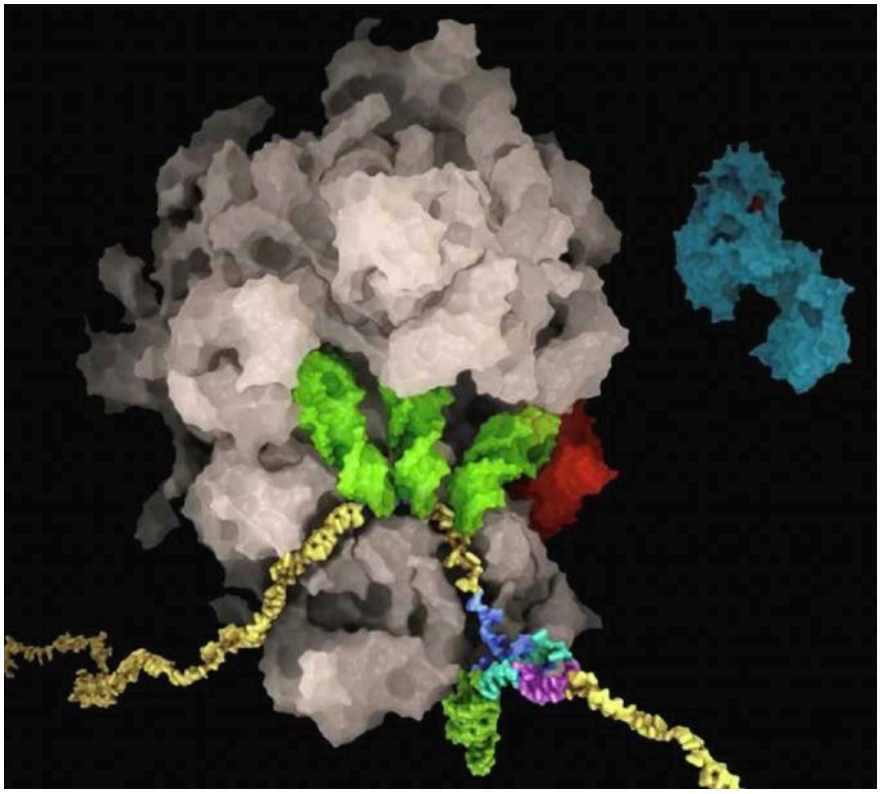
By Cryo-EM, they solved the structure of the 80S ribosome paused at the SARS-CoV-2 frameshifting site. For this complex, the core of the ribosome was resolved at 2.2.-Å resolution, which is the highest resolved structure of a mammalian ribosome to date. Consequently, as an extra bonus of the study, the high-resolution information allowed for the visualization of many protein and rRNA modifications never directly seen before but known from other methods. *"We were stunned by the resolution obtained since we did not push for this methodologically. The resolution results from the ribosome adopting a strained conformation when trapped at the frameshifting site."* explains Scaiola.

From previous research, it was known that the frameshifting occurs only when the ribosome interacts with the slippery frameshifting site and as well with a pseudoknot structure downstream. These interactions could be visualized in the structure. To the big surprise of the researchers, specific interactions also take place between the ribosomal exit tunnel and the nascent chain being translated when the ribosome hits the frameshifting site. Two of the amino acids of the nascent chain that interact with the ribosome show a high degree of conservation among Coronaviruses, despite their location in an unstructured region of the viral protein.

Based on their structure, the researchers performed follow-up biochemical and cellular experiments. With the combination of the applied experimental approaches, the researchers obtained a detailed molecular and mechanistic understanding of this intricate process essential for Coronavirus replication. So far, similar ribosomal frameshifting has not been reported for native cellular transcripts, so that its inhibition by drugs could be a way to treat all types of Coronavirus infections. *"Based on how conserved this process is, as well as the interactions between the ribosome, the viral RNA and the nascent chain, it would be difficult for the viruses to evolve mutations, making them resistant to drugs targeting the frameshifting event."* says Scaiola.

In the literature, two compounds targeting the pseudoknot were proposed to inhibit the frameshifting, and only one of them was tested in infected cells. When the researchers treated SARS-CoV-2 infected African green monkey cells with the two compounds, viral replication was decreased up to four orders of magnitude without apparent cellular toxicity caused by the compounds. Interestingly, contrary to previous reports, only one of the two had a dose-dependent effect on frameshifting, while the other seems to reduce viral replication through a different mechanism. Although these compounds are not potent enough for being used as drugs, the results provide a starting point for drug development.

Bhatt, Scaiola et al. (2021) Science 372(6548), 1306-1313 (Open Access)



The RNA (yellow) of the SARS-CoV-2 virus forms a pseudoknot structure (multicolored, bottom right) which leads to a shift in the reading frame of the ribosome (brown).(Still image from molecular animation by Said Sannuga kindly provided by the authors.

Molecool

Kosmos RNA
Cosmos ARN
Cosmos RNA

As a central new instrument for public communication, we have created MOLECOOL – COSMOS RNA. The trilingual website (German, French and English) serves to communicate knowledge and engage in a dialogue with the public. The content of the website is centered around the RNA molecule. It is regularly updated with contemporary articles about recent research highlights from NCCR labs, explanatory articles about RNA biology and articles covering societal discussions of RNA-related research findings. Together with the associated social media channels, the website aims to generate and satisfy genuine interest in basic research in the main target group – high school and university students and curious adults of any age.

Share & distribute with your friends, colleagues, students, acquaintances, social media networks…
Feedback & contributions. If you have suggestions for content on Molecool or feedback to the website, please contact office@nccr-rna-and-disease.ch.

#NCCRWomen Campaign

Meet the NCCR women!

To celebrate the 50th anniversary of women obtaining the right to vote in Switzerland, all NCCRs currently in place across the whole country joint forces to release a series of videos showcasing women working in science. The videos are targeted at girls and boys of school and undergraduate age. The videos aim to influence the stereotypes of how people picture a scientist and to give insights into the everyday life of scientists. Each NCCR will host a week where they will publish several videos covering multiple scientific disciplines, from 8th of March (International Women's Day) until 31st of October 2021 (50th anniversary of the women's vote in Switzerland).

The NCCR RNA & Disease shot five movies in Bern and in Zurich to portrait several female researchers at different career stages. The following five portraits were published in the week from June 28th – July 2nd, 2021 on YouTube:

June 28, 2021:
Portrait of **Valeriia Volodkina,** PhD student from the Meister Group, Institute of Cell Biology, University of Bern.
Watch on YouTube.

June 29, 2021:
Portrait of **Magdalini Polymenidou,** Professor at the Department of Quantitative Biomedicine, University of Zurich.
Watch on YouTube.

June 30, 2021:
Portrait of **Jasmin van der Heuvel** (Postdoc), **Kerstin Dörner** (PhD student), **Claudia Gafko** (PhD student), **Chiara Ruggeri** (PhD student) from the Kutay Group, Institute of Biochemistry, ETH Zurich.
Watch on YouTube.

July 1st, 2021:
Portrait of **Nancy Carullo,** Postdoc from the Mansuy group, Brain Research Institute of the University Zurich and Institute for Neuroscience of the ETH Zurich
Watch on YouTube.

July 2nd, 2021:
Portrait of Stefanie Jonas, Professor at the Institute of Molecular Biology & Biophysics, ETH Zurich.
Watch on YouTube.

Follow the **#NCCRWomen campaign** on YouTube, Instagram, Twitter and the hashtag #NCCRWomen to meet women who work in research in Switzerland!

Announcements

People

We would like to welcome Maria Hondele and Anne Spang, who are both Professors at the Biozentrum of the University of Basel, as new associate members. The Hondele lab studies the formation and function of membraneless organelles with a focus on ones that are associated with RNA processing. The Spang lab researches intracellular localization and transport of RNA and proteins.

We congratulate Ulrike Kutay on receiving the ETH ALEA award, which *"honors leaders who enable advanced and innovative working conditions and who promote and support actively the reconciliation of work, family and avocational engagement"* and Nenad Ban for being elected as a member of the US National Academy of Sciences.

Congratulations also to associate members Anne Spang for becoming a member of the German National Academy of Sciences Leopoldina and Karsten Weis for being elected EMBO Member.

Support grants

Please visit our webpage for more information on the Lab exchange program, the Mobility grants and measures in Equal Opportunities.

Translational grants

An NCCR RNA & Disease Translational fellowship was awarded to Özgür Genç to conduct a translational project in the laboratory of Peter Scheiffele (University of Basel).

Upcoming events organized or supported by the NCCR RNA & Disease

> NCCR Seminar Series Autumn Semester 2021 & Spring Semester 2022
  - **Anne Willis** (University of Cambridge, UK) 1.11.2021 Bern & 2.1.11.2021 Zurich
  - **Gisela Storz** (National Institutes of Health, Bethesda, USA) 14.3.2022 Bern & 15.3.2022 Zurich
  - **Geraldine Seydoux** (Johns Hopkins University, Baltimore, USA) 2.5.2022 Bern & 3.5.2022 Zurich
  - **Amy Pasquinelli** (University of San Diego, USA) 16.5.2022 Bern & 17.5.2022 Zurich

More seminar speakers to be announced.

> Scientifica – Zurich Science Days, September 3–5, 2021

> Bench2Biz workshop 2021: The next Bench2Biz workshop will take place as a virtual event during five afternoons in November 2021. More information on the Bench2Biz website.

> 22nd Swiss RNA Workshop, January 21, 2022, Bern

NCCR RNA & Disease Internal Events

> "You are Full of Power" workshop – Career Navigation for Women, September 16–17, 2021, Bern
> 6th NCCR RNA & Disease Annual Retreat, January 24-26, 2022, Engelberg

Jobs

PhD program in RNA Biology
The next application deadline is December 1, 2021.
Find out more on the PhD program website.

Check the jobs's section of the NCCR RNA & Disease webpage for other openings.

Join our new LinkedIn NCCR RNA & Disease Current Members & Alumni Group and follow us on LinkedIn and Twitter!

IMPRINT

The National Centres of Competence in Research (NCCR) are a research instrument of the Swiss National Science Foundation

NCCR RNA & Disease
Phone: +41 31 631 38 12
office@nccr-rna-and-disease.ch
www.nccr-rna-and-disease.ch

Office Bern
University of Bern
Departement of Chemistry and Biochemistry
Freiestrasse 3, CH-3012 Bern

Office Zürich
ETH Zürich
Institute of Biochemistry, Biochemie II
HPP L14, Hönggerbergring 64
CH-8093 Zürich

UNIVERSITÄT BERN

ETH zürich

FNSNF
SWISS NATIONAL SCIENCE FOUNDATION