THE MESSENGER

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NCCR
RNA & Disease

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

Dear colleagues

This Messenger issue features another interview with an expert on a Covid-19 related topic. The interview is with Dr. Jasmin Barman, who was involved in setting up the SARS-CoV-2 PCR diagnostics at the Triemli Hospital in Zurich. She and her colleagues are associate members of the NCCR and long-standing collaborators of ours on researching new treatment options for the rare genetic disease Erythropoietic Protoporphyria (EPP).

EPP causes patients to develop extreme pain after exposure to light, which is nicely explained on any number of internet sites. Through which, Jasmin, an EPP patient herself, discovered the cause of her suffering. However, internet pages and scholarly readings in my experience cannot provide a complete picture of how such diseases impact patients' lives on numerous levels. For instance, I was once stunned when Jasmin explained to me her discomfort when travelling on the new generation of Zurich public buses with their intense LEDs as interior lighting. Hearing about these daily experiences gives you a different perspective and added urgency when researching a given rare disease.

The "good" news, in the context of the several thousands of rare diseases, is that most of them have a genetic cause and thereby, an associated target for the development of new therapeutics. Furthermore, as novel technologies such as RNA-based drugs come on-line, they are often able to bring new treatment approaches with higher rates of clinical success than for conventional drugs. Our NCCR is a strong driver of such innovative research.

Jonathan Hall
KTT Delegate
NCCR RNA & Disease

Interview Jasmin Barman-Aksözen

"Now, I am like everybody else and long for the sunshine."

Interview: Veronika Herzog & Dominik Theler

In this interview, Jasmin Barman-Aksözen gives us insights into Covid-19 testing, her career, rare diseases in general and especially into Erythropoietic Protoporphyria (EPP). She is herself an EPP patient and conducts research on the disease and treatments for it.

How did the pandemic affect the EPP community?
Surprisingly, I – like most other patients – feel that the restrictions caused by the pandemic did not affect us too much, probably because we for the most of our lives were used to living in a kind of lockdown. The imposed restrictions resemble the normal life of people with untreated EPP. However, we were anxious whether the situation would affect access to our treatment. Luckily, we were able to receive the treatment throughout the pandemic, so far.

How did the pandemic affect you personally?
Working in a diagnostics lab, I was part of the team that introduced and conducted the PCR testing for SARS-CoV-2. We had established PCR diagnostics for other pathogens already, and this method was not too hard to adapt to SARS-CoV-2, but the challenge during the initial phase of the pandemic was to obtain sufficient reagents and plastic consumables. We even got some support from our partner labs at the ETH and the University of Zurich, which donated their stocked materials. Luckily, we never had to send SARS-CoV-2 samples away due to lack of materials, but sometimes the timelines were very tight.

Were you ever close to getting exhausted?
Luckily not. In the beginning of the pandemic, I supported our team of biomedical scientists because we had to introduce night and weekend shifts to ensure timely delivery of the results. It was an interesting experience to be back to the bench and using a pipet, and this with such a purpose. Because of the lockdown other projects were stopped, but at some point, I had several hours of cumulated overtime which I however could compensate during the next months. Later, we hired dedicated people for the SARS-CoV-2 testing so that the regular team could focus on the standard tests performed in the hospital. The burnout danger was not the only challenge, but to protect the team against infections and other hazards. We had to ensure that the standard diagnostic testing is available for non-Covid patients, whom the hospital had to continue treating during the pandemic.

Were you approached with technical questions from the public or even persons doubting the validity of PCR testing or discussing applied CT values?
As lab members, we were directly affected by the pandemic but nevertheless kind of behind the lines. We are happy, when asked, to explain the test, its reliability, and what for example a CT value means. Seeing the reality in the hospital and at

Interview Jasmin Barman-Aksözen

the same time people publicly claiming that the pandemic is all fake news makes me sad. It also shows a high demand for knowledge distribution. All we can do is to share our knowledge, hope that people will listen and become convinced by the facts.

Going away from the pandemic and back to you, the weather just went from sunny to rainy: Are you happy about that?
No! Although you are right, a few years ago – before I started treatment with afamelanotide which reduces the sensitivity to light in people with EPP – this was my favourite weather. Now, I am like everybody else and long for the sunshine.

What made you become a scientist? Was this connected with your disease?
I was fascinated by biology and wanted to work with plants or animals. When I heard about DNA in school, it was clear that I wanted to become a biologist. Initially, I wanted to study photosynthesis to use the energy of the sunlight through genetic engineering to do something for the environment. I did not have my diagnosis at that time and did not know that I got severe pain when being exposed to light because of my DNA. So, I did not connect these two things.

How did you find out about your disease?
When I was 27 years old and about to finish my biology studies, I could not sleep because I had again symptoms. So, I searched the internet: I found this new Wikipedia article named *"erythropoietic protoporphyria"* and thought: *"Wow, this is the description of my life."*

With Drs. Google and Wikipedia are patients getting their diagnosis earlier?
Yes, this is an excellent effect. We now have more patients getting their diagnosis in their early childhood. It makes a huge difference for them and their families to know what they suffer from.

You did not get your diagnosis in childhood: How was this for you and your family?
We realized that my symptoms came from exposure to sunlight, so my parents adapted our family life to that. For example, we did not go to the beach in our holidays but to the forest, where it would be shadowy enough for me to spend time outside. Later, I tried to cover myself with clothes and used an umbrella when outdoors. However, this was not sufficient because you cannot cover your entire body, for example the face, and light comes from the sides and is reflected from white surfaces and water. So, I often ended up with pain and symptoms at the end of the day. When getting older, I started to make excuses, for example that I needed to study for an exam, and therefore could not join to go to the swimming pool and would only come later to the cinema. I was hiding the problem because it was complicated to explain it to others. They said: *"but today it is not that sunny"*. They did not understand that as a patient with EPP, you are in danger as soon as there is some light.

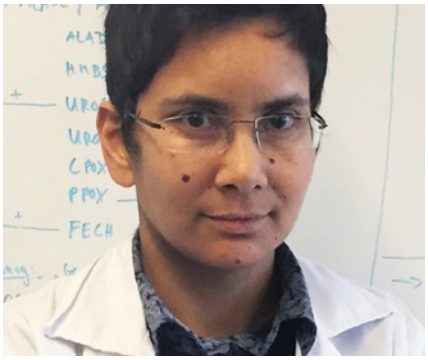
Would it have been easier if you already had your diagnosis as a child?
Definitely. Such a cool, long, and complicated name as *"erythropoietic protoporphyria"* is something of value and different from saying that you just cannot stand the sunlight. We teach the newly diagnosed kids to say the whole term and not only the abbreviation *"EPP"* and not describe it as sunlight allergy, which is incorrect. When the kids say the full name, they gain respect from their peers and have a different standing. Also, with the diagnosis, they for example can explain that the visible and not the UV part of light is the problem. Before my diagnosis, many physicians advised me to take this or that brand of sunscreen. When I always came back and said that this had not worked, they stopped believing me at some point.

Despite sometimes even having visible phototoxic burn reactions like red hands and open burn wounds, some people accuse you of malingering or telling you that your symptoms are psychosomatic. I was even two weeks in the hospital after being sent to the North Sea, which was supposedly beneficial for persons suffering from allergies. But since EPP is not an allergy, this did not help. On the contrary, I got a lot of burns due to the exposure to sunlight there. Still, they did not believe that it was the sunlight causing the symptoms and did not even write it into my medical records. As a consequence, I started to avoid persons, including health care professionals, who did not believe me.

What are symptoms specific for EPP important for a physician to recognize?
Extreme pain of the exposed body parts with minor or absent visible symptoms after exposure to sunlight or strong artificial light, occurring from early childhood on. These should be the warning signs to think about an EPP.

Why did you decide to study your own disease for your PhD?
I contacted the patient organization that posted the Wikipedia article that I mentioned before. It was fantastic for them that it re-

"I found this new Wikipedia article named 'erythropoietic protoporphyria' and thought: Wow, this is the description of my life."



Jasmin Barman-Aksözen

Biography

Jasmin Barman-Aksözen studied Molecular Biology, Biochemistry and Plant Sciences at the University of Heidelberg, Germany. For a PhD project on gene expression in EPP she moved to Zurich to the Institute of Laboratory Medicine at the Triemli Hospital and was supervised by Elisabeth Minder and Xiaoye Schneider-Yin. After her PhD, she became Head of Clinical Chemistry laboratory at the same institute. In 2015, she, her PhD supervisor Elisabeth Minder and former Head of the Institute and the current Head of the Institute Xiaoye Schneider-Yin became associate members of the NCCR RNA & Disease.

Institute for Laboratory Medicine of the Triemli Hospital

"It was fantastic for them that it resulted in an additional diagnosis after only two weeks of posting."

Interview Jasmin Barman-Aksözen

sulted in an additional diagnosis after only two weeks of posting. So, they asked me to present my case at a symposium attended by European experts. I mentioned during my presentation that I am about to finish my molecular biology studies. After the talk, several experts in the area approached me and asked if I wanted to join them and work on an EPP research project.

I started to consider this option and reflected a lot on it because studying your own condition might be too personal. However, it is also very fascinating from a molecular biology viewpoint that one base in your DNA makes the difference whether you have a disease that so profoundly affects your life. I visited a couple of labs and decided to join the group of Elisabeth Minder at the Triemli Hospital in Zurich. She and Xiaoye Schneider-Yin had the idea to influence the underlying splice defect in the RNA found in over 95 % of people with EPP with a small oligonucleotide so that the cells produce more of the missing enzyme. I thought this was such a fantastic project, and we are still pursuing this goal through a great collaboration with the group of Jonathan Hall and Daniel Schümperli.

"When working with patient cells, I did not think I was manipulating a small EPP patient in the dish."

Your fears that it would be too close to study your own disease, did they materialize?
If you use molecular and cell biology techniques, your research is pretty abstract. When working with patient cells, I did not think I was manipulating a small EPP patient in the dish. However, a huge advantage of being a patient of the disease was that I sometimes quickly could test ideas using my own blood samples.

Still, in science, you can receive harsh criticism when applying for grants or peer review of publications. Did you have problems with that not taking it too personally?
Not for my basic research. In research, I consider myself a scientist and not a patient, and as long as the criticism is scientifically based, that is fine for me and of course even can improve my work. However, I do have a problem with apparently unscientific assessments which I unfortunately had to experience in our work regarding afamelanotide, the first effective therapy for preventing phototoxic burn reactions in EPP.

Can you tell us more about afamelanotide, its development as an EPP treatment, your role in this process and the issues you encountered?
Afamelanotide is a small peptide developed in the 1980ties at the University of Arizona, which is an analogon of the endogenous α-Melanocyte-stimulating hormone. Like the natural hormone, afamelanotide induces eumelanin production and has strong anti-inflammatory properties. An Australian biotech developed an implant formulation that enabled the medical use of afamelanotide and tested it as a protection against melanoma in people that underwent kidney transplantation. In 2006, a Swiss biochemist and patient with EPP suggested to my PhD supervisor, Elisabeth Minder, to try afamelanotide as a treatment for EPP. He thought that increasing the pigment load of the skin could create a physical barrier against the visible light. As a consequence, less visible light penetrates the skin and reaches the blood vessels, it the sites of the pain and burns. However, we now know that the anti-inflammatory properties are more important for the protective effect of afamelanotide than the increase in pigmentation.

Elisabeth Minder indeed started the first clinical trial in patients with EPP before I joined the team. The data from the trials was encouraging and because I knew many of the trial participants personally trough the patient community, I got the impression that the treatment was effective. People not being able to tolerate more than 10 minutes of sunlight exposure that lived in hiding from all outdoor activities now on their weekends went to hiking tours in the mountains and told me that sunshine felt warm and pleasant on their skin! Being a PhD student in the group investigating afamelanotide I could not join the clinical trials myself, but I was involved for example with the trial design and the development of assays for testing the safety of afamelanotide like tests for detecting anti-drug antibodies. In addition, I supported the development of patient reported outcome measures like the disease specific Quality of Life questionnaire and more recently the "phototoxic burn tolerance time". Because EPP is an ultra-rare disease with at that time no effective therapy in place, no

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EPP

Erythropoietic Protoporphyria (EPP) is a rare genetic disease, with an estimated number of 80 patients in Switzerland. The disease arises from mutations in the Ferrochelatase gene, which encodes for the last enzyme of the heme synthesis pathway. Ferrochelatase incorporates the iron and its absence leads to the accumulation of the accumulation of the precursor molecule Protoporphyrin IX (PPIX). PPIX reacts with the visible spectrum of light creating reactive oxygen species which damage the tissue and lead to extreme pain in the body parts that were exposed to light. Prolonged exposure to light can lead to swellings and category two skin burns. Approximately 10% of the patients suffer from liver damage due to the PPIX accumulation, which can become life-threatening.

One therapy (Afamelanotid) is available which reduces the sensitivity to light but does not tackle the PPIX accumulation. One of the NCCR RNA & Disease projects aims at developing new treatment options for EPP. One option would be based on a splice-switching oligonucleotide, which could be due to the genetic nature of the disease, be used to treat an estimated 95% of EPP patients. Most patients carry one inactive allele of the Ferrochelatase gene and the other allele is a common variant in the population, of which a large fraction of the transcripts are mis-spliced due to a single base substitution activating a cryptic exon acceptor site leading to the incorporation of a stop codon. Persons who carry two copies of the variant allele do not display symptoms.

Link to the Institute for Laboratory Medicine of the Triemli Hospital which is the Swiss Reference Center for Porphyrias.

Interview Jasmin Barman-Aksözen

Jasmin Barman as a child with swellings in the face the day after sun exposure. Picture by Arun Barman & provided by Jasmin Barman.

experience or tools were available to measure treatment effects or Quality of Life. Then, after the trials, I had finally the change to test the drug myself, and this truly was and is a life changing experience for me.

Because of my background as a scientist having the disease, I had the chance to act as a patient representative during the approval proceedings at the European Medicines Agency (EMA) and to participate in a meeting with the FDA. However, there was a substantial disbelief by these regulatory authorities that the drug works for EPP and doubts that the disease is severe enough to be treated. This personally affected me as a patient and a scientist. It was very hard to deal with these disbelief and doubts, especially because the results of the four randomised controlled clinical trials did show statistically significant improvements, for example in the time patients under treatment spent in direct sunlight without suffering from painful burns compared to the placebo treated control group. I knew, without approval, the new life and freedom we patients gained through the treatment were threatened to be taken away. In the end, we managed to convince the regulatory bodies of the severity of EPP and the benefits of the treatment, and the huge difference it makes for us patients and the committees decided to approve Afamelanotid. Currently, Afamelanotid is approved in Australia, the EU, and the US for the treatment of adults with EPP. However, it is an ongoing struggle to convince every national authority to also reimburse the treatment, and many patients still remain without access.

What is still lacking is a formulation for children, who are the most affected group of patients with EPP. Currently, work is ongoing to develop a scaled formulation that can be adjusted to kids' body weight, but it is not ready yet. The treatment of children is an urgent medical need, and I am convinced that all adult EPP patients would forego their own implants and give it to children, if it would be possible.

Besides being a patient advocate and researcher of your disease, you also engage in public outreach through science slams: How did you get into this?

I once witnessed a science slam at a conference and when there was one organized at ETH in 2012, I just had to participate. A science slam is a completion between usually four to six researchers presenting their own research work in only 10 minutes in an educational and at the same time fun way. They are allowed to use all means and techniques, for example power point presentations, singing, dancing, even experiments with the audience – basically everything that helps to make their point. In the end the audience judges who did the best job in presenting their research. I was used to explain the disease and was invited several times by patient organizations to present my research results. Therefore, I thought *"how hard could it be to do a science slam on EPP"*? I can tell you, the first time was really exciting for me, but I also felt the support of the other science slammers which is a great community of people dedicated to their research and to sharing it with the public. For me, science slams turned out to be a great and fun way to raise awareness for EPP and the other the porphyrias. And they work! I got feedback from physicians that they re-considered the symptoms of some of their patients and finally could properly diagnose them after attending one of my science slams. Also, slams give you a lot of media attention, which was beneficial when we were trying to raise awareness for the reimbursement of Afamelanotid.

"I have never regretted going public with my condition."

Still, you are probably the most publicly known Porphyria patient in the German-speaking part of Europe, having been featured in major newspapers, magazines, and TV. Have you ever regretted this outing, and are there disadvantages associated with it?

I have never regretted going public with my condition. It was not the plan to become famous for being a patient with EPP but rather for my research. However, when the EMA first did not want to invite patients for the approval hearings of afamelanotide. The president of the Swiss patient organization asked me to tell my rather unique story to the public: I discovered my condition, researched it, and was involved in developing the first effective treatment for it that changed my own life. And after weighing the pros and cons, I decided to go for it which was also a cool experience. I think that without going public, we likely would not have the treatment today

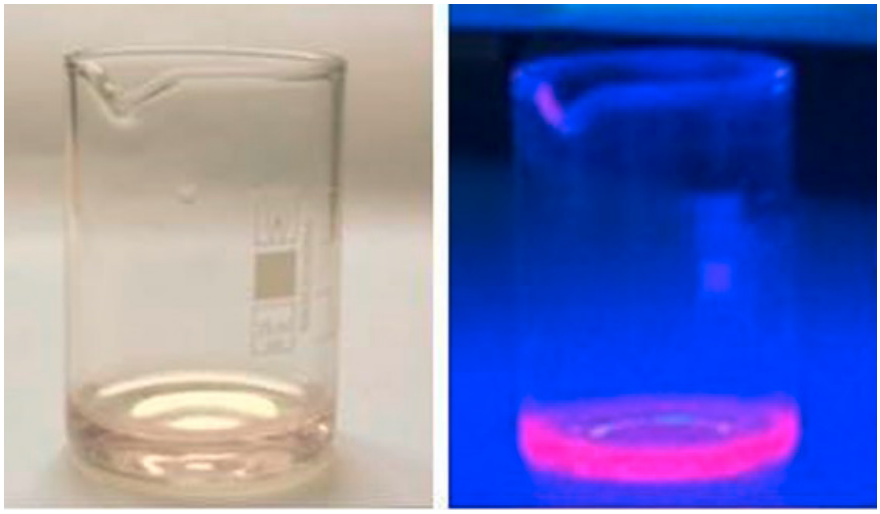
"As a consequence, I started to avoid persons, including health care professionals, who did not believe me."

What was a key discovery moment during your scientific career?

Until 2015, EPP was understood as a disease in which the phototoxic heme precursor protoporphyrin IX accumulates in the maturing erythrocytes because of a reduced activity of the last enzyme in the heme synthesis pathway, Ferrochelatase. However, I discovered that there in addition is an upregulation of Aminolevulinic acid synthase 2 or ALAS2, which is the first and rate-limiting enzyme of the heme biosynthesis pathway in the erythrocytes. This discovery could lead to causative treatment options for EPP. I recently participated in a nice project with Francois Halloy from the lab of Jon Hall who used repurposed drugs in a cell culture model of EPP to decrease the synthesis of protoporphyrin by reducing the amount of intracellular Glycine, one of the substrates for the ALAS2 reaction. Indeed, a biotech from the US already plans to conduct a phase II trial in patient with EPP testing one of the substances characterised by us! If successful, this would be the first causative treatment for EPP and therefore could also protect against the hepatotoxic effects of protoporphyrin IX.

After your PhD, how did the focus of your professional life develop?

I did my PhD in the Institute for Laboratory Medicine of the Triemli Hospital. Later, I became the Head of the Clinical Chemistry



Fluorescence of protoporphyrin IX (PPIX) in 1.5 M HCl upon excitation with visible blue light (LED). Image kindly provided by Jasmin Barman.

laboratory at the Triemli which performs diagnostics for many different diseases, which is very interesting. Besides this, I am currently taking courses in regulatory affairs, health economy and health technology assessment at the ETH and the Zurich Applied Sciences University in Winterthur. The aim is to combine both worlds, the generation of high-quality data in rare diseases by basic research and optimised trial designs, and to support the approval and reimbursement of safe and effective drugs.

"There are presently more awareness and possibilities for research as well as several new therapies available."

Have things improved over the last years for rare disease patients?
Yes, definitely! There are presently more awareness and possibilities for research as well as several new therapies available. For example, at the University of Zurich, there now is a University Research Priority Program for rare diseases called ITINERARE – Innovative Therapies in Rare Diseases. Also, university centres for rare diseases are being appointed in Switzerland, which people can turn to when they have symptoms that could so far not be explained. And the Triemli hospital was recently officially appointed the Swiss reference centre for porphyrias!

It is estimated that half a million people in Switzerland suffer from a rare disease, which is impressive and equals the number of patients with diabetes in Switzerland. However, this patient population is split between around 8000 rare diseases. The field is enormous and research-wise very interesting because you can discover so many new mechanisms by studying rare diseases. Moreover, many rare diseases are linked to more common diseases and the knowledge gained from these rare diseases can also help to better understand and treat the more common ones.

What are today's unmet needs of the rare disease community?
How long do you want this interview to be, an additional two hours? There are needs at every step of the path. The unmet need includes the awareness for the diseases, lack of treatments, lack of access to diagnostics, experts in the field, and medical knowledge, and issues for example in insurance coverage. There are common patterns in the issues encountered by many patients with different rare diseases, and one can learn from a success story for one condition for others. That is one aspect why coordinated initiatives such as ITINERARE are essential.

Interview conducted on October 4, 2021.

Research Highlights

Ribosomal ubiquitin-like fusion protein: Same same but different?

Dominik Theler

In most eukaryotes, one ribosomal protein of each subunit is initially translated as an in cis linear fusion with a ubiquitin moiety. These N-terminal ubiquitin moieties are cleaved off during ribosome biogenesis. The cleaved-off ubiquitin is a major source of cellular ubiquitin, which can be conjugated to other proteins. It can only be speculated about why such a link between ribosome biogenesis and the ubiquitin-based protein degradation system exists. It could serve to coordinate the cellular protein synthesis rate with degradation.

Another type of ribosomal fusion protein, FUBI-eS30, exists in humans and other holozoan organisms. The N-terminal half of this fusion protein consists of the ubiquitin-like protein FUBI, while the C-terminal eS30 is a ribosomal protein being part of the mature 40S subunit. The Kutay lab, which has a long-standing interest in ribosome biogenesis, set out to investigate FUBI-eS30.

For the classical ribosomal ubiquitin fusion proteins, it is known that only the ribosomal protein parts of the fusion proteins, but not ubiquitin, are part of mature ribosomes. This becomes already apparent when looking at ribosome structures because uncleaved ubiquitin moieties would sterically impair the ribosome function. The same holds true for FUBI. *"When working on ribosome biogenesis, I enjoyed the availability of detailed structural data. It gives you a visual idea of what you are working with and helps to view your hypotheses from different angles,"* says Jasmin van den Heuvel, first author of the study published in eLife and former PhD student in the Kutay lab.

Like ubiquitin, FUBI contains at its C-terminus a double glycine motif. Mutation of this motif hinders cleavage of the ubiquitin moiety from its *in cis* fusion protein. The researchers created two FUBI-eS30 mutants based on previous literature in which the diglycine motif was mutated. These constructs also contained a tandem StHA tag at the C-terminus of eS30, and as a control, a wild-type construct with the same C-terminal tag was created. Compared to the wild-type construct, the diglycine mutant proteins were not cleaved in cells. Also, their expression led to decreased 40S and polysome levels. At the same time, increased 60S levels were observed, which was previously reported when 40S production was hindered. Through further experiments, they could dissect that the non-cleavable mutants led to a defect in the late 40S maturation in the cytoplasm.

The researchers performed mass spectrometry with the previously mentioned constructs to identify the protease responsible for cleaving FUBI from eS30. Two known deubiquitinases (DUBs) were enriched in the pull-downs of the uncleavable constructs compared to the wild-type construct. Depletion experiments with siRNAs showed that only one of them, USP36, was responsible for cellular FUBI cleavage. Using CRISPRi for the depletion of endogenous USP36, only a

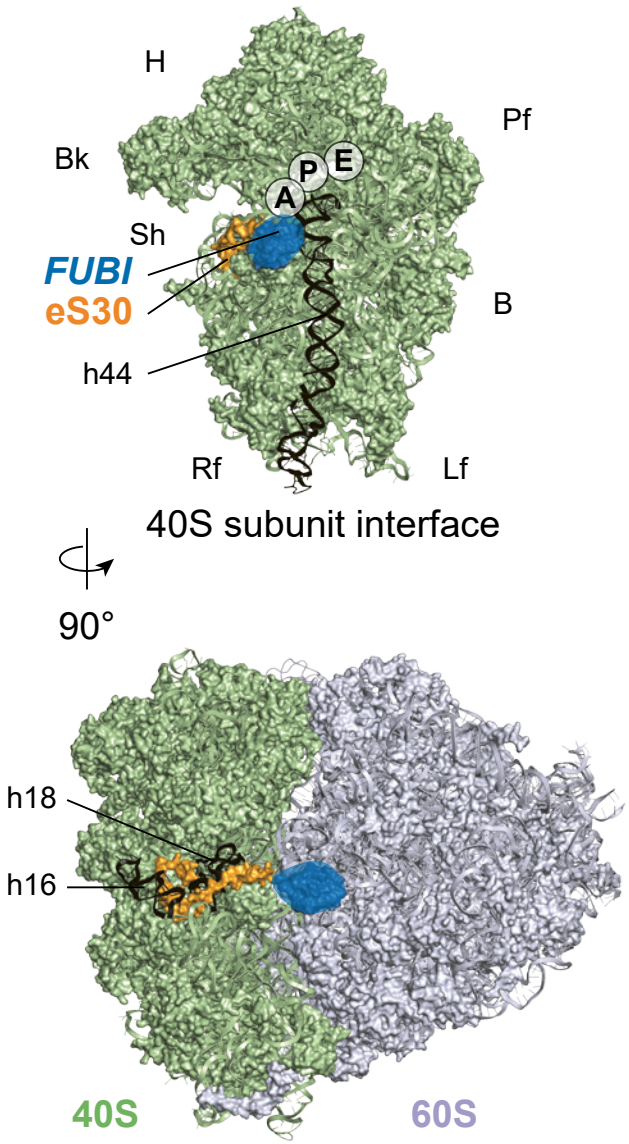


Figure from van den Heuvel et al. (2021) eLife published under a CC BY 4.0 license.

wild type USP36 containing construct, but not one with a catalytically inactive mutant, could revert the accumulation of uncleaved FUBI-eS30 in cells. Also, they showed that USP36 cleaves FUBI-eS30 *in vitro*. Based on these experiments, USP36 is a bispecific enzyme that can cleave ubiquitin and FUBI off its fusion protein.

Like for ribosomal ubiquitin fusion proteins, the FUBI moiety of the fusion protein with the ribosomal protein eS30 needs to be cleaved by a DUB to create functional ribosomes. An open question is if FUBI, like ubiquitin and some other ubiquitin-like proteins, is used to modify other proteins. Although it cannot be ruled out that other enzymes are involved in the cleavage of FUBI-eS30, USP36 seems to be the key player in this process. Interestingly, the oncogenic transcription factor Myc is one of the known proteins that USP36 deubiquitinates and thereby stabilizes. Thus, USP36 could be an interesting target for cancer drug development. *"I was initially skeptical whether anti-cancer drugs can be used to target fundamental processes such as ribosome biogenesis or nuclear transport without killing too many healthy cells in an organism. But the first drugs targeting these processes seem to show that at low doses, cancer cells are susceptible to these drugs while normal cells can tolerate such doses,"* says Ulrike Kutay, last author of the study and Full Professor at the Institute of Biochemistry at ETH Zurich.

Publication:
van den Heuvel et al. (2021) eLife, 10, e70560 (Open Access)

NMD-sensitive transcripts catalogued at unprecedented detail in human cells

Veronika Herzog

Cells harbour a variety of quality control mechanisms to prevent the production and accumulation of harmful proteins. The quality control mechanism nonsense-mediated mRNA decay (NMD) is a translation-dependent RNA degradation pathway that targets mRNAs with premature stop codons as well as many mRNAs that encode full-length proteins. NMD is essential for correct embryonic development in all mammals and plays an important role in tumors and genetic diseases such as cystic fibrosis. In a new study published in the journal *Genome Biology,* the labs of Oliver Mühlemann (University of Bern) and Mihaela Zavolan (Biozentrum in Basel) joined forces to gain new insights into this quality control mechanism by mapping endogenous NMD targets on a global scale at unprecedented detail using Nanopore sequencing *(Karousis et al., Genome Biology, 2021).*

NMD was initially discovered to degrade aberrant mRNAs that carry premature termination codons (PTC) arising from mutations or errors in RNA processing, such as transcription or splicing. Later studies that characterized NMD substrates on a transcripome-wide scale revealed that NMD not only removes PTC-containing transcripts, but also controls the overall expression level of many mRNAs encoding full-length proteins. These studies sparked the efforts to understand what features of transcripts beyond the presence of an early stop codon render transcripts NMD-sensitive.

In healthy cells, where NMD is active, it is difficult to detect defective mRNAs because they are rapidly degraded after production. To detect NMD targeted transcripts, Karousis and colleagues depleted the key NMD factors UPF1, SMG6 and SMG7 either individually or in combination in HeLa cells. Under these conditions, NMD-sensitive transcripts accumulate and can be detected by high-throughput sequencing.

Previous methods for detecting NMD-targets in cells have relied on short-read sequencing techniques (Illumina) generating short sequences that are later computationally reassembled into whole transcripts. However, in this gigantic puzzle, many pieces cannot be unambiguously assigned to a specific transcript. Evan Karousis, first author of the study, explains that they applied Nanopore sequencing – a rather new method that decodes full-length mRNAs in one piece. "*This allows to overcome the limitation of short-read sequencing technologies as each detected mRNA can uniquely be assigned to an mRNA variant*", Karousis said. *"In addition, Nanopore sequencing allows for the discovery of new RNA isoforms, among which many are NMD targets"*. The combination of long-read Nanopore sequencing with short-read Illumina sequencing enabled the researchers to create a comprehensive and high-fidelity catalog of NMD targeted RNAs in human cells.

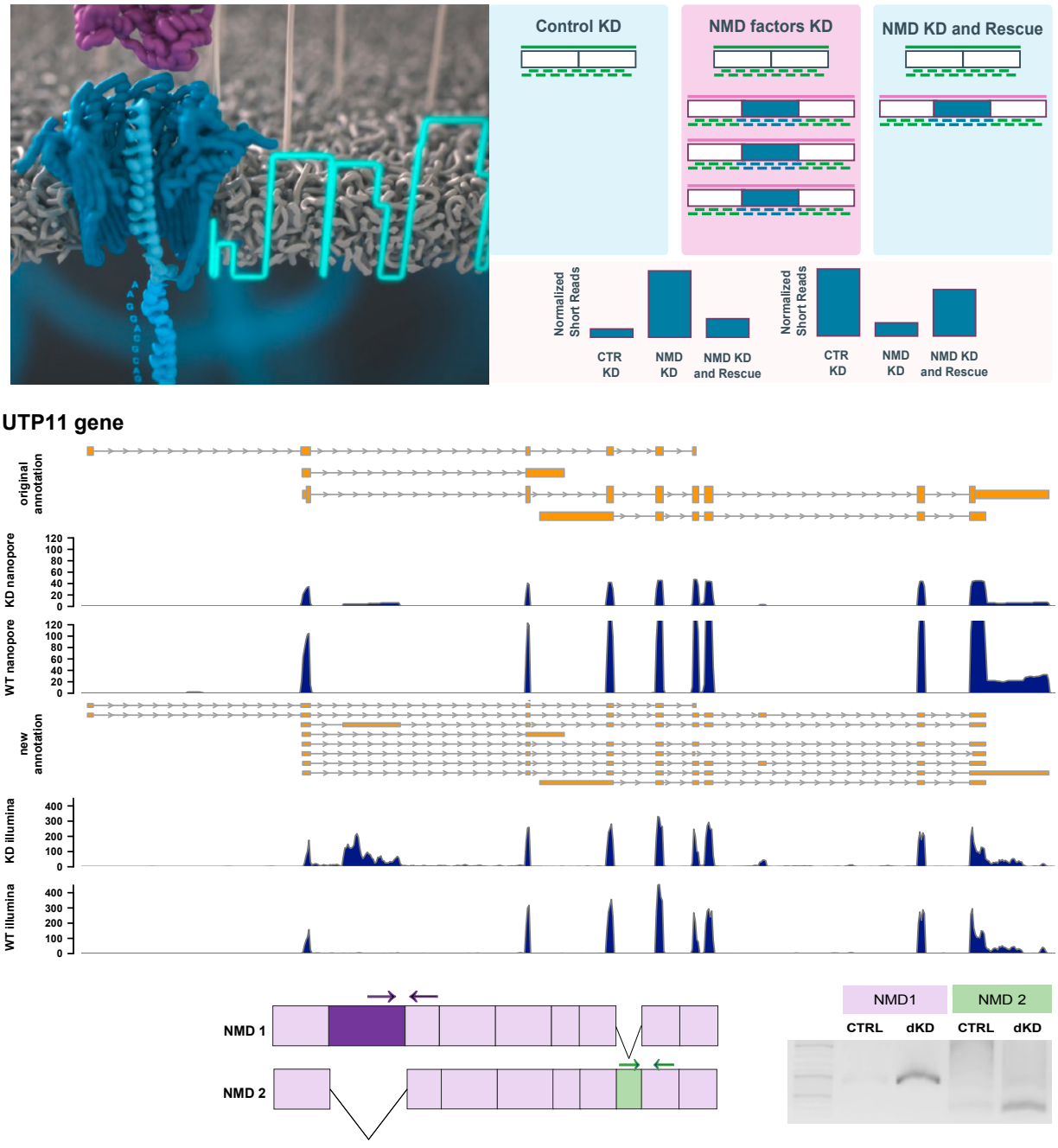
Using this catalog of NMD-sensitive transcripts, the researchers tried to identify features that would allow *ab initio* prediction of NMD targets. They could confirm a

Research Highlights

central role of exon junctions in the 3' UTR for rendering a transcript sensitive to NMD. Surprisingly – and in contrast to previous findings –, the length of the 3' UTR did not correlate with NMD sensitivity for mRNAs with the stop codon in the last exon. And finally, the authors showed that NMD targets both canonically and non-canonically spliced mRNAs – supporting the notion that NMD serves not only as a quality control mechanism to remove faulty spliced transcripts but also as a general mechanism of regulating gene expression.

The importance of these findings is evident: The accurate classification of NMD sensitive transcripts and the identification of NMD-inducing features has the potential to provide insights into the degradation mechanism. "*If certain mRNAs accumulate that are degraded in healthy cells by NMD quality control, this can contribute to the development of tumors, as is the case in gastric cancer, for example*", Oliver Mühlemann explains. So if we understand how the quality control can distinguish defective from correct mRNAs, this knowledge will contribute to the development of new therapeutic approaches for diseases in which quality control is impaired.

Publication:
Karousis et al. (2021) Genome Biology, 22 (1), 223 (Open Access)



Top left image with permission from Oxford Nanopore. Two other figures from Karousis et al. (2021) Genome Biology published under a CC BY 4.0 license.

Scientifica – Zurich Science

The NCCR RNA & Disease at the Scientifica 2021

Dominik Theler

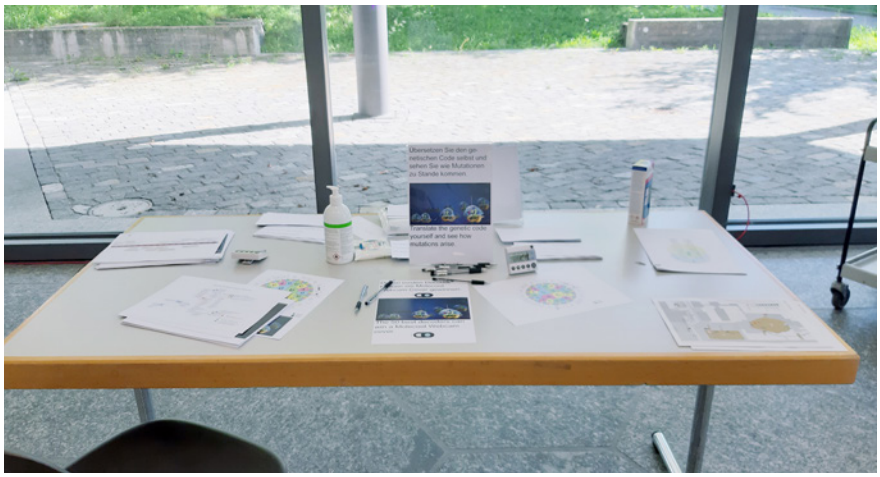
On September 4 & 5, 2021, the Scientifica – Zurich Science Days took place, during which ETH Zurich and the University of Zurich showed the public trough stands and several event formats their research. The NCCR RNA & Disease participated for the fourth time to the Scientifica with a stand, which was for this edition on *"mRNA-Translation – a central process for mRNA vaccines and SARS-CoV-2"*.

At the NCCR's booth the visitors could in the form of a challenge translate mRNA into the corresponding first 20 amino acids of the SARS-CoV-spike protein. The winners of the challenge were sent a Molecool webcam cover after the Scientifica. Furthermore, they could learn more about translation and the NCCR research on ribosome inhibition by NSP1 and the frameshifting occurring during translation of the SARS-CoV-2 genome using a translation puzzle, a 3D printed ribosome & NSP1 protein and also inspecting the structure with a 3D visualization system. As well, they could play the RNA eater game, have a look at a BSL3 protection suite, explore the Molecool website and watch the *"No Denial"* short movie as well as the five short portrait films of NCCR female researchers filmed in the context of the NCCRWomen campaign.

The NCCR was one of three selected stands at Hönggerberg to be featured in the Facebook livestream. Click here to see Adrian Bothe from the Ban lab presenting the NCCR stand during the Facebook Live stream.

We would like to thank very much the persons involved in the NCCR's booth as well as the organizers and staff of the Scientifica!

The researchers presenting the NCCR RNA & Disease stand on Sunday.



RNA translation challenge at the Scientifica 2021.

3D visualization system, 3D printed models and translation puzzle.

Participating Groups

Nenad Ban	*ETH Zurich*
David Gatfield	*University of Lausanne*
Stefanie Jonas	*ETH Zurich*
Ulrike Kutay	*ETH Zurich*
Oliver Mühlemann	*University of Bern*
Volker Thiel	*IVI & University of Bern*

Concept & coordination:

Dominik Theler	ETH Zurich and University of Bern

Announcements

People

We congratulate **Stefanie Jonas** (Institute for Molecular Biology & Biophysics, ETH Zurich) and **Roger Geiger** (Institute for Research in Biomedicine, USI, Bellinzona) who were selected as EMBO Young Investigators as of January 2022!

Congratulations to **Michael N. Hall** (Biozentrum, University of Basel) on receiving an honorary doctorate from the Hebrew University of Jerusalem, Israel!

We would like to welcome **Yves Barral** (Institute of Biochemistry, ETH Zurich), **Emanuela Felley-Bosco** (Thorax Surgery Clinic, University Hospital Zurich), **Donald Hilvert** (Laboratory for Organic Chemistry, ETH Zurich) and **Raphaëlle Luisier** (IDIAP Research Institute, Martigny) as new associate members of the NCCR RNA & Disease!

The **Barral lab** studies asymmetric cell division, cellular compartmentalization and aging using yeast as model organism. In this context, they investigate the role of RNA and RNA binding proteins in these processes.

The **Felley – Bosco lab** researches the molecular oncology of mesothelioma including the role of RNA editing and its use as a therapeutic target, as well the roles of non-coding RNAs and post-transcriptional gene regulation.

The **Hilvert group's** research interest is chemical biology and protein engineering. The protein engineering part concerns enzymes as well as encapsulation and delivery systems. They developed a protein cage, which can be used for example to deliver RNAs up to the size of siRNAs.

The **Luisier group** integrates several types of datasets e.g clinical, genomic and imaging data for the analysis of complex diseases such as neurodegenerative diseases. For this type of analysis, the group develops statistical and machine learning methods.

New Co-Delegate for KTT

Ramesh Pillai (University of Geneva) became the new Co-Delegate for KTT succeeding Rory Johnson, who moved to the University College Dublin. We would like to thank Ramesh Pillai for volunteering to become the new KTT Co-Delegate and Rory Johnson for all his efforts for KTT and taking the initiative to create this position.

New PhD and Postdoc Representatives

We welcome Maria Escura Pérez (Allain lab, ETHZ) as the new PhD student representative and Esther Griesbach (Chao lab, FMI) as the new Postdoc representative of the NCCR RNA & Disease. They succeed Esteban Finol (Allain lab, ETHZ) and Rajani Gudipatti (Grosshans lab, FMI) who stepped down as representatives.

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

Follow Molecool on Facebook and Instagram.

New "Equal Opportunities" Measure: Parental Leave

The NCCR RNA & Disease reserved funds to cover the salary of PhD students or postdocs during six weeks of parental leave. Fathers (or partners in same-sex couples) of a newborn child are eligible to apply for the parental leave. The six weeks come on top of the regular two weeks Swiss paternity leave.
Check the equal opportunities website for more information.

Swiss RNA Workshop 2022

After last year's cancellation, the Swiss RNA Workshop took again place in 2022 on January 21. The workshop was conducted virtually due to the pandemic situation. The scientific program consisted of keynote lectures by Stefanie Jonas and Volker Thiel, 11 short talks selected from abstracts and nearly 40 poster presentations.

Support grants

Please visit our webpage for more information on the Lab exchange program, the mobility grants and measures in equal opportunities.

Upcoming events organized or supported by the NCCR RNA & Disease

> NCCR Seminar Series Autumn Semester 2021 & Spring Semester 2022
  - **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
  - **John Mattick** (UNSW Sydney, Australia) 6.5.2022 Zurich
  - **Amy Pasquinelli** (University of San Diego, USA) 16.5.2022 Bern & 17.5.2022 Zurich

NCCR RNA & Disease Internal Events

> 6th NCCR RNA & Disease Annual Retreat, March 21-23, 2022, Engelberg

Jobs

PhD program in RNA Biology
The next application deadline is July 1, 2022.
Find out more on the PhD program website.

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

IMPRINT

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