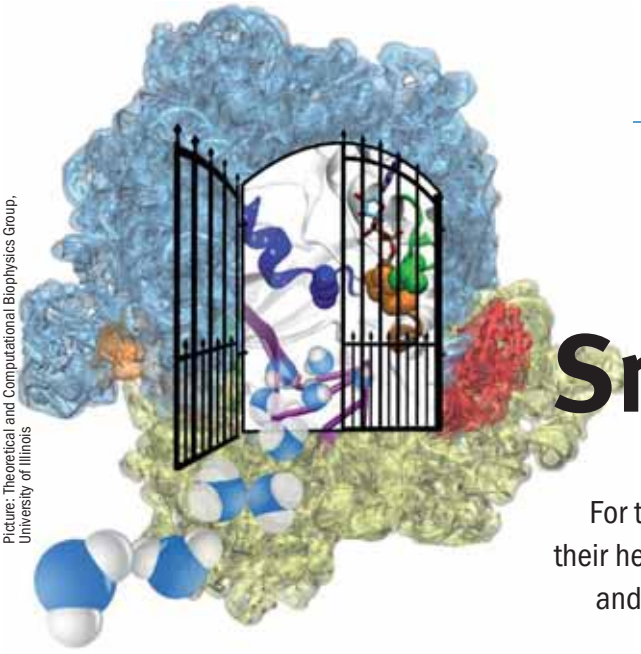

Picture: Theoretical and Computational Biophysics Group, University of Illinois

Molecular microbiology in Munich, Germany

Smooth Translation

For the last 30 years, researchers around the world have been scratching their heads over the elongation factor P mystery. What is the protein's target and how does it act on it? Now, almost simultaneously, two groups have worked it out. One of them is the group of Kirsten Jung.

Elongation factor P (EF-P) is a multi-faceted bacterial protein known to be vital for cell proliferation, survival, stress resistance and virulence. Its target and mechanism of action, however, have remained shrouded in mystery. That was until a team of scientists at the Ludwig-Maximilians-Universität in Munich (LMU), Germany, discovered the missing piece of the puzzle and published their find in *Science* (339(6115):82-5.

Where it all started: the Cad module

Our story begins with the study of the Cad module, a lysine-dependent acid-resistance system, in the laboratory of Kirsten Jung. Jung had studied biochemistry and during her postdoctoral research became interested in membrane proteins. In 2004, she took over the chair in Microbiology at the LMU and has since been working on understanding how bacteria utilise receptors to perceive environmental information. Her group has long been working on the Cad module, which responds to three different stimuli: low pH, lysine and cadaverine concentration. The latter being a foul-smelling compound produced during the decomposition process of dead animals. The Cad module consists of CadC (a membrane-integrated sensor and transcriptional regulator) that regulates induction of the cadBA operon, which, in turn, encodes for CadB (lysine/cadaverine antiporter) and CadA (lysine decarboxylase).

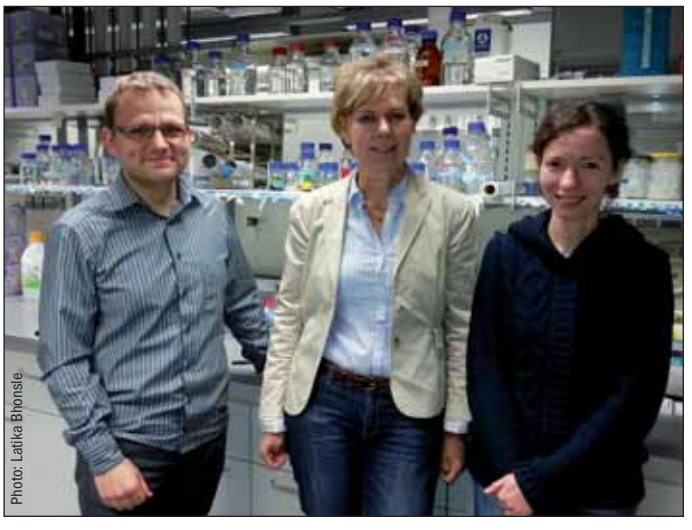

Photo: Latika Bhonsle

Solving bacterial mysteries: Jürgen Lassak, Kirsten Jung and Susanne Ude (from left to right)

1st clue: An aminomutase called Yjek

As Jung explains, "At first, we thought this was a very simple system but soon we realised that there were multiple components involved. So, we did a transposon mutagenesis with the intention of finding new players. And there was only one hit and this was yjeK". Jung knew that this gene coded for YjeK, a lysine 2,3-aminomutase (an enzyme that modifies lysine by shifting the amino group from the alpha to the beta position) and thought that it might be involved in the lysine-sensing function of the Cad module. She then entrusted Susanne Ude, a PhD student, with the task of elucidating the role of YjeK in this scenario.

Ude had studied biology in Munich and switched to Freiburg for her diploma studies. During an internship at the University of Oxford, in the group of Andrew Spiers, she discovered her interest in microbiology and decided to carry out her diploma thesis in the field of cyanobacteria and non-coding RNAs, with the group of Wolfgang Hess. She then joined the Jung lab as a PhD student and began work on YjeK.

Ude saw that the Cad module was not responsive to stress (negative phenotype) on knocking out yje. All her experiments provided pieces to a puzzle that did not seem to fit together. Until she came across a theoretical paper that would change the course of their research.

2nd clue: EF-P enters the plot

"The paper suggested that YjeK and YjeA (EF-P-lysine34-lysine ligase) were important for the modification of EF-P," says Ude. This was the same EF-P that had baffled scientists for more than 30 years. EF-P has long been a subject of interest to the scientific community as it is a conserved protein with orthologs in archaea and eukaryotes, and is known to bind to ribosomes and stimulate peptide bond formation. However, nobody knew what the target of EF-P was, or how EF-P acted on this target. Jung and Ude decided to probe further. "We then looked at a delta yjeA mutant and found that this, too, had a CadA negative phenotype," recalls Ude. They now wanted to test their hypothesis with an EF-P deletion mutant and this was when postdoc Jürgen Lassak joined the project.

Lassak's scientific journey began as a student of biology at Tübingen, followed by a diploma in the group of Yuen-Tsu Nicco Yu at the Max Planck Institute (MPI) of Development Biology. He then spent two years studying antibiotics and resistance mechanisms as a scientist at the Leibniz Institute for Natural Product Research in Jena, before he started his doctoral research at the Thormann group at the MPI in Marburg. A strong interest in regulatory mechanisms and signal transduction encouraged him, in due course, to apply for a postdoctoral position at the Jung laboratory.

3rd clue: An 'impossible' mutant

Obtaining the EF-P deletion mutant proved to be very difficult and the researchers were further discouraged by scientific literature suggesting that an EF-P mutant was impossible to make, as it was coded by an essential gene. Surprisingly, however, one such mutant was available in the Keio collection (an *E. coli* K-12 single-gene knockout mutant resource located in Japan). Without much ado, they called for it and tested for the CadA phenotype. To their delight, the EF-P deletion mutant also lacked CadA activity, clearly confirming the connection between EF-P and CadC. The trio had found the target. It was now time to determine how EF-P acted on it.

4th clue: Discovering the target motif

Lassak elucidates the next step, "We then came up with the idea of generating translational fusions between CadC variants of different lengths and LacZ (β-galactosidase). We observed an EF-P dependent translation when we made a certain part of the cadC open reading frame precede the lacZ. This begged somehow for a motif between an EF-P dependent and independent chimera." This motif turned out to be a polyproline cluster. "When we exchanged the prolines, translation became EF-P independent and this got us very excited as we had come up not only with a target but also a signature sequence," he adds triumphantly.

5th clue: Ribosomal stalling

"We now had the knowledge that EF-P is needed for the translation of CadC and we also knew the motif but what we still needed was biochemical proof. We were really lucky that our neighbouring group, that of Daniel Wilson working in the Gene Center, had an established set up for transcription translation experiments. So we contacted them, provided all our data and realised then that they, too, had been looking for the target of EF-P," recounts Jung. The *in vitro* experiments from Wilson's laboratory showed that CadC translation only proceeded until the polyproline cluster, at which point the ribosome stalled, and this stalling was alleviated in the presence of EF-P. With their elegant methodology, the scientists had found the much searched-for answer to the EF-P mystery.

Coincidentally, around the same time, a research team headed by Marina Rodnina in Göttingen, Germany had come to the same conclusions using a kinetics-based approach. A chance meeting at the LMU generated a lot of excitement when they realised that their research findings complemented each other and they decided to publish these as back-to-back stories.

Complementary findings

The implications of these results are far reaching. In *E. coli*, there exist about 100 proteins (out of a total of 4,000) that bear the polyproline signature and are, hence, dependent on EF-P for translation. Out of the ten proteins (of diverse function and localisation) analysed in Jung's study, each showed an EF-P dependent translation.

The Jung, Lassak and Ude findings, are, however, not only important for prokaryotes but also for eukaryotes, especially in the context of virology and cancer research. Lassak cites an example, "The regulator of virion expression (Rev) of the human immunodeficiency virus (HIV) is the one and only viral protein that contains a polyproline stretch". Ude adds, "A eukaryotic homolog of EF-P is also a tumour marker, as one of the isoforms is highly expressed in tumour cells". The knowledge that EF-P is indispensable for the translation of such pivotal players could very well open up new research avenues for therapeutic targets.

In addition, the trio discovered that EF-P based translational regulation of the Cad module also plays a role in fine tuning the copy number of the receptor. "We determined that, on average, only three to five copies of the receptors are found per cell in bacteria, and that is a very low number. We have two hypotheses: either this low copy number drives phenotypic heterogeneity and not all cells respond to a particular stress stimulus but perform other tasks or, such a translational regulation process might help to insert certain membrane-integrated proteins and hence have a functional importance," explains Jung. In the near future, the group plans to shed light on these implications of their findings.

Hypothesis fully confirmed

This particular paper strikes a chord with the scientific community, not only because of the strong scientific data it presents but also because of the elegant methodology that the authors decided to follow. In the words of Kirsten Jung, "From my point of view, this was one of those stories that you would always like to have in the lab because we proposed something, did the experiments and the results came out exactly as expected."

Latika Bhonsle