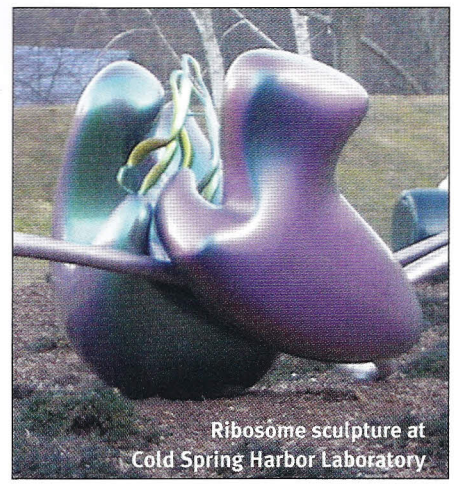
Structural Biology in Munich, Germany

Witnessing the Birth of Nascent Proteins

Ribosomes are cells' protein producing factories. Their main function is to translate the genetic instructions encoded in the form of messenger RNA into the protein building blocks of living tissue; a process aptly known as translation. A lot is known about translation biochemistry but still lacking is a structural understanding of the events associated with this process.

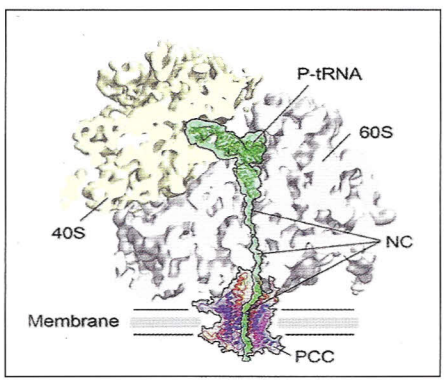


Ribosome sculpture at Cold Spring Harbor Laboratory

The group under Roland Beckmann, based at the Gene Centre of the Ludwig-Maximilians-University (LMU) in Munich, focuses on unearthing this very information. In two recent papers (T. Becker *et al., Science* 326: 1369-73 and B. Seidelt *et al., Science* 326: 1412-5), they have used cryo-electron microscopy (cryo-EM) and single particle analysis to obtain information at sub-nanometre resolution; that has enabled them to, "see the nascent protein chain as it is being synthesized by the ribosome".

Helping proteins enter their new life

The first paper addressing the interaction between the emerging nascent chain and the protein conducting channel sheds light on protein translocation across membranes and mainly presents data from the post doctoral research of Thomas Becker.



The Sec complex binds to the exit site of the ribosome

In higher organisms like mammals, some newly synthesized proteins are routed from the ribosome to a tubular network called the endoplasmic reticulum (ER), which facilitates protein folding and cellular transport. The Sec complex (in humans: sec61, in yeast: Ssh) is a well conserved protein-conducting channel (PCC) involved in this process. Not only does it help proteins traverse the ER membrane but also aids their insertion into cellular membranes.

Roland Beckmann has been fascinated with the function and architecture of the Sec Complex since his days as a postdoc in the lab of Nobel prize winner Günther Blobel at Rockefeller University, New York. "Being able to see such complexes at work are very fascinating," he says. Using cryo EM data he was the first to suggest a binary model of co-translational translocation for the protein complex in 2001 (R. Beckmann *et al., Cell* 107, 361-72). In humans, Sec61α and β form an end to end funnel, with Secγ acting like a plug (Ssh1, Sbh2 and Sss1 in yeast).

"The active structure was suggestive of a mechanism where the nascent chain may pass through, pushing the plug as it emerges into the ER," says Beckmann. However, the precise interactions that governed this process and the oligomeric conformation adopted by the active PCC was still unknown. In order to answer these questions, it was imperative to achieve a higher resolution and establish a suitable *in vitro* system where this phenomenon could be studied.

Small observations, big implications

The task was given to Thomas Becker, who joined Beckmann's lab in August 2001 and devoted his PhD and post doctoral research to this cause. He spent long hours analysing potential substrates and testing reconstitutions; until he finally hit upon the optimal combination. His recent paper draws conclusions both from the yeast and mammalian Sec complex; revealing the structural interactions between the translating ribosome and Sec61/SecY complex of the endoplasmic reticulum (ER) during co-translational translocation.

The Sec complex was detergent solubilised from the ER (tests confirmed that lipid molecules stabilizing the protein complex were not lost during this process) and then reconstituted with a translation extract that carried a truncated message. "A truncated message simply means that the mRNA in this case does not carry a stop codon and, hence, the translated nascent chain is not released from the ribosome," elaborates Becker. Whole complexes were flash frozen and examined by cryo-EM. "The image analysis of many individual particles, randomly oriented in the ice layer, permits construction of an average three-dimensional model of the complex at high resolution," explains Beckmann.

Pushing the resolution

This study has shown, for the first time, how the nascent chain is accommodated within the PCC during co-translational translocation. Becker explains, "We were able to visualise the alpha helical structure of the Sec complex at a resolution close to 6 Å and observed that the PCC binds to the exit site of the ribosome. The nascent chain (NC) could be followed from its growing end through the ribosome into the PCC," he adds. Additionally, they could also confirm that, in agreement with X-ray structure, one copy of the trimeric Sec complex is sufficient for activity, a finding that puts to rest an age-old debate.

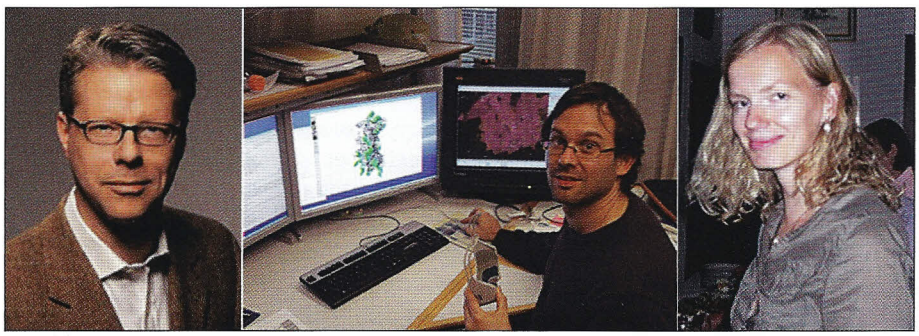
What can we expect in the future from the Sec complex story and Thomas Becker? "We plan to test different constructs in a similar setting and also visualise the nascent chain as it passes through the PCC. To achieve this, we are working to push the resolution further to the sub-nanometre range," says Becker. "The next step will be to visualise these interactions in the context of an intact membrane," he adds. In fact,

a PhD student within the group has made progress in this direction and will soon publish his findings.

While in the lab, Becker regularly works in parallel with other lab members to address different aspects of the Sec complex. In addition, he also shoulders teaching responsibility and conducts lectures and practical courses at the LMU for undergraduates. "Against popular advice, I did my PhD and postdoc within the same research group," he tells. However, this persistence paid off not only in the form of landmark papers but also overall job satisfaction. "I like my boss and the working group," he confesses with a smile; "and I also have the unique opportunity of working in a fascinating research area, using the most advanced tools available."

Speed bumps

In the second study, PhD student Birgit Seidelt, in collaboration with the Thomas Steitz team at Yale University (Nobel Prize in Chemistry 2009), elucidated how structural conformations within newly synthesized proteins may regulate protein synthesis.



From left to right: Roland Beckmann, Thomas Becker, Birgit Seidelt

"Stalling ribosomal models are often used to visualise the nascent chain," says Beckmann. "These allow us to examine a precise stalling point and are more likely to catch the nascent chain in a defined position." For the recent study, a cryo EM model of a bacterial ribosome stalled during the translation of the TnaC leader peptide (of the tryptophanase operon), yielded insights into the site-specific interactions of the extended nascent peptide chain within the ribosome exit tunnel. Axel Innis from the Steitz group provided the preparation, whereas Birgit Seidelt performed the cryo-EM analysis.

They observed, at 6 Å resolution that the nascent chain within the ribosomal tunnel had an extended conformation that interacted specifically with surrounding nucleotides and ribosomal proteins. Furthermore, these interactions were relayed to the peptidyltransferase centre of the ribosome; thereby preventing the binding of release factors. "This observation could explain the inhibition of translation termination," concludes Beckmann. Apart from that, the study also added weight to the fact that nascent chains can adopt distinct conformations during orientation within the ribosomal exit tunnel.

"In the near future, we would like to study the downstream characteristics of nascent chains in more detail," explains Beckmann. "We are planning to study other stalling peptides and see if they follow similar mechanisms of regulation, and also how nascent chain properties like hydrophobicity may affect these interactions," he elaborates.

After completing his postdoc in New York, did he not think of continuing research in the United States? "The German system, in my opinion, is underestimated," states Beckmann. The teaching effort is substantial, yes, but the degrees of freedom and the safety are very different when compared to the US. I am in a nice position and am not strictly bound," he concludes.

Pushing for the next generation

After five years in Berlin, Beckmann finally moved base from Berlin to the LMU, Munich in 2006 as it offered him and his group the possibility of high end single particle cryo EM in a vibrant life science research environment. "We were supported generously by the university and the government, and now have the possibility to work with the Titan Krios, the latest in a line of new generation electron microscopes. This is the basis that we are currently engaged in bringing the resolutions closer to 4 Å as this would give more reliable data to study connectivity and secondary structure involved in ribosomal processes."

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