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Nucleic Acid Science – The Excitement of Discovery

Annual Symposium of the Chemical Society Zürich CGZ, Zürich, October 26, 2007

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Abstract: The Chemical Society Zürich held its annual symposium on frontiers in nucleic acid science at the Eidgenössische Technische Hochschule Zürich. The conference successfully bridged nucleic acid chemistry and biology, with topics including new developments in DNA nanotechnology, mechanisms of gene transactivation by a left-handed form of DNA, chemical catalysis by RNA enzymes, state-of-the art developments in mass spectrometry of RNA–protein complexes and structural analysis of gene transcription by a large multisubunit RNA polymerase enzyme.

Keywords: DNA nanostructures · Mass spectrometry · Ribozyme · RNA polymerase III · Z-DNA

The Event

This meeting was the sixth Annual Symposium of the Chemical Society Zürich in its current format, alternating annually between ETH and University Zürich. The symposium took place in Zürich at ETH Hönggerberg. It was hosted by the President of the Chemical Society Zürich (Dr. *I. Berger*, ETH) and jointly organized by the authors (Institute of Molecular Biology and Biophysics) and Prof. *F. Thoma* (Institute of Cell Biology) of ETH Zürich. Close to 250 participants came to the event.

The Annual Symposium was generously sponsored by the Institute of Molecular Biology and Biophysics of ETH, the Institutes of Biochemistry and Inorganic Chemistry of University Zürich, the Molecular Life Sciences Program Zürich (MLS), the PhD Student Lecture Series (ETH), Prof. U. Suter from the Institute of Cell Biology (ETH), VentureKick and numerous industrial sponsors including Biorad, Bruker, Brunschwig, Clontech-Takara, Invitrogen, Merck-Serono, Microsynth, Novartis, Roche, Redbiotec, Syngenta, Tecan and VWR International AG.

CGZ Annual Symposium 2007: Christiane Schaffitzel (Questor), Christoph Bieniossek (Actuary), Imre Berger (President), Julia Fritz-Steuber (Former President), Andres Jaeschke, Alexander Rich, Nadrian C. Seeman, Carol V. Robinson, Christoph W. Müller, Fritz Thoma (Co-organizer)

Keynote Lecture

The Keynote Lecture, chaired by the President, was given by Prof. ***Alexander Rich***, pioneer of molecular biology and currently the William Thompson Sedgwick Professor of Biophysics at MIT (Cambridge, MA, USA). His lecture was entitled ‘Gene Transactivation by Z-DNA Binding Proteins’.

Prof. Rich presented an overview of the events that commenced with the discovery of a left-handed form of DNA in his laboratory almost thirty years ago.[1] This form became known as Z-DNA due to the zigzag arrangement of the diester linkages in

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Jack Dunitz, Alfred Eschenmoser, Alexander Rich

Alexander Rich and Imre Berger

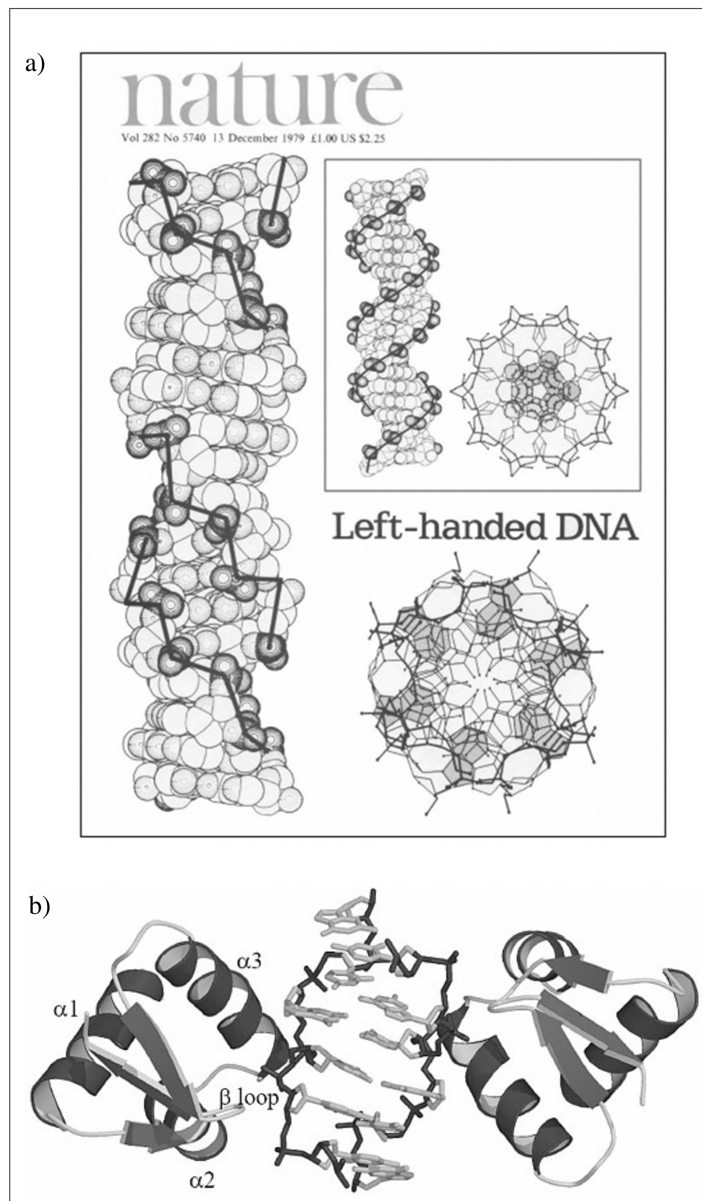

Fig. 1. a) Z-DNA on the front cover of Nature magazine in 1979. b) Structure of the complex between Z-DNA and the Zα domain of DLM-1 protein.

the sugarphosphate backbone (Fig. 1a). The discovery of Z-DNA provoked intensive research into the possible physiological function of this unusual DNA conformer in the cell. Prof. Rich described the physico-chemical properties that set apart Z-DNA from the right-handed B-form and can be used for its detection *in vitro*, and the stabilizing effect of negative supercoiling on Z-DNA within regions of alternating purine and pyrimidine bases in chromosomes during transcription and the unwrapping of DNA in nucleosomes caused for example by chromatin remodeling proteins. He pointed out that sequences which are favourable to Z-DNA formation are present near transcription start sites of genes.

A breakthrough in the quest for a biological function of this unusual DNA was the discovery of Z-DNA specific proteins in Prof. Rich's laboratory in the 1990s.[2] Prof. Rich presented the X-ray structure of the specific complex between Z-DNA and the Zα domain of the editing enzyme dsRNA adenosine deaminase, ADAR1.[3] Sequence homology searches based on tyrosine, tryptophane and prolin residues essential for binding revealed many other proteins specific for Z-DNA, such as E3L of Vaccinia virus and human DLM-1 (Fig. 1b). This set the stage to develop a powerful biological assay for Z-DNA binding *in vivo* by measuring the lethality of mice following intra-cerebral inoculation with Vaccinia virus containing E3L variants with domains exchanged from ADAR1 or DLM-1, or, alternatively, variants of ADAR1 domains containing mutations impairing Z-DNA binding. The observed lethality *in vivo* correlated with the strength of Z-DNA binding measured *in vitro*.[4]

Prof. Rich presented results of experiments elucidating the mechanism of E3L activity and its correlation to Z-DNA binding involving co-infection of HeLa cells with constructs expressing E3L deletion variants and constructs with various enhancer elements inserted in front of a luciferase reporter. This demonstrated the binding of E3L to the promoter of activated genes upon infection and the importance of the Z-DNA binding activity of E3L in its transcriptional activation effect on human genes including interleukin 6 (IL-6). Prof. Rich concluded his lecture by delineating the transactivation effect of E3L on the well-studied human c-myc promoter which contains three well characterized Z-DNA forming regions.

As an intriguing outlook, Prof. Rich alluded to recent results from Taniguchi and co-workers who identified the Z-DNA binding protein DLM-1 as a cytosolic receptor and an activator of innate immune response,[5] raising exciting questions of how a Z-DNA binding protein with similar properties as E3L may help induce interferon production.

DNA Nanotechnology

Prof. ***Nadrian C. Seeman*** (New York University, USA) presented an exciting lecture of 'DNA: Not Merely the Secret of Life'. Prof. Seeman's research is focused on the use of DNA to built artificial nanometric devices and arrays for supramolecular templated assembly, illustrating the architectural power of using DNA as the perfect nanotool.[6] By using DNA double-crossovers and multi-stranded structures with anti- and mesojunctions, Prof. Seeman's laboratory created superstructures from DNA including cubes (Fig. 2), truncated octahedra, borromean rings and 2D crystalline arrays. Prof. Seeman illustrated his successes in constructing two-dimensional patterns from DNA by showing atomic force microscopy images of nanometer scale objects of DNA with templated shapes, indicating that expanding this approach into three dimensions may become possible in the near future.

In the second section of his lecture, Prof. Seeman focused on DNA nanomechanical devices. He presented a molecule that utilizes the salt-dependent B–Z transition of DNA to create a nanoswitch that emits fluorescent light upon addition of salt.[7] A more complex DNA nanomechanical machine from Prof. Seeman's laboratory even acts as a translation device that selects DNAs in a similar fashion to ribosomes selecting tRNAs for message combination. Prof. Seeman concluded his equally informative as entertaining lecture by demonstrating his novel 'walking biped' made of DNA.

RNA Diels-Alderase

The final speaker of the first session was Prof. ***Andres Jaeschke*** (University of Heidelberg, Germany). Prof. Jaeschke's work focuses on ribozymes designed for catalyzing Diels-Alder reactions in a stereoselective manner. Catalytic RNA molecules are powerful and highly selective catalysts. By using a product-based selection regime, Prof. Jaeschke discovered a RNA molecule that catalyses carbon–carbon bond formation in a Diels-Alder reaction, which is one of the most important reactions in synthetic chemistry.

Using *in vitro* selection by SELEX, Prof. Jaeschke's team generated from a combinatorial RNA library a molecule that catalyzed carbon–carbon bond formation by a Diels-Alder reaction with multiple turnovers.[8] This RNA molecule consists of 49 nucleotides, and accelerates the Diels-Alder reaction by about 20,000-fold in an enantioselective fashion. 'Chemical mutagenesis' of substrate and RNA was used to delineate the molecular basis of the catalytic reaction.[9]

Prof. Jaeschke presented the X-ray crystal structure of the molecule he selected (Fig. 3), which resembles the greek letter λ.[10] Comparison with known protein Diels-Alderases revealed striking similarities in the architecture of the binding pocket. Prof. Jaeschke concluded this session by summarizing architectural principles of the Diels-Alderase ribozyme and the function of individual molecular features during substrate conversion, and providing an outlook on how the reaction can be regulated by altered ribozymes evolved to rely on an effector molecule.[11]

Nadrian Seeman

Andres Jaeschke

Frontiers in Mass Spectrometry of Large Multisubunit Complexes

The second session of the Symposium was chaired by Dr. *C. Schaffitzel*, Questor of the Chemical Society. The first speaker was Prof. ***Carol V. Robinson*** (University of Cambridge, UK). Modern mass spectroscopy (MS) is an indispensable tool in the study of protein interaction networks. MS allows for the analysis and characterization of the entire protein repertoire of cells. Recent technological advances, such as ion mobility-MS (IM-MS), have estab-

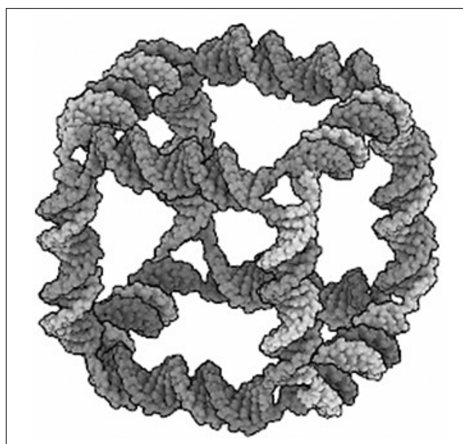
Fig. 2. Nanocube made of DNA

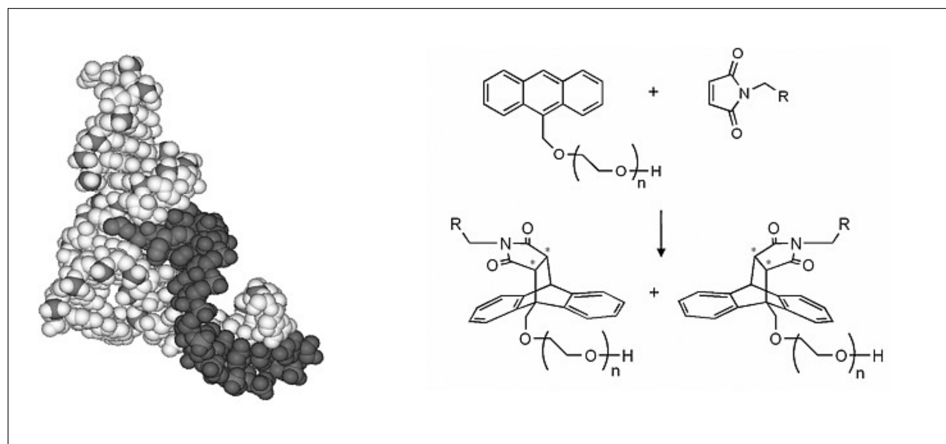
Fig. 3. λ-Shaped RNA Diels-Alderase and the reaction it catalyzes

lished mass spectrometry as a powerful methodology for structural biology applications. Prof. Robinson showed IM-MS experiments deducing the stoichiometry and overall topology of protein complexes. Even the arrangement of protein subunits within a given multiprotein assembly can be determined in this way.

Prof. Robinson explained the principles of a modern mass spectrometer for structural characterization of large multiprotein complexes. A prerequisite for any structural studies by MS is that the solution-phase structure can be maintained in the gas phase. This can be achieved using nanoelectrospray ionization of the sample. Further, using collision-induced dissociation, very large complexes can be selectively dissociated by collision with neutral gas atoms. Activation occurs when a portion of the kinetic energy of an ion is converted into internal energy during each collision event. If sufficient internal energy is accumulated, this may lead to subsequent dissociation of this ion. Subsequently, the subunits or subcomplexes are analyzed in the time-of-flight (ToF) instrument. With the current instruments, it is possible to analyze virus capsids and entire ribosomes (Fig. 4) with a molecular mass of 2.5 Mda.[12]

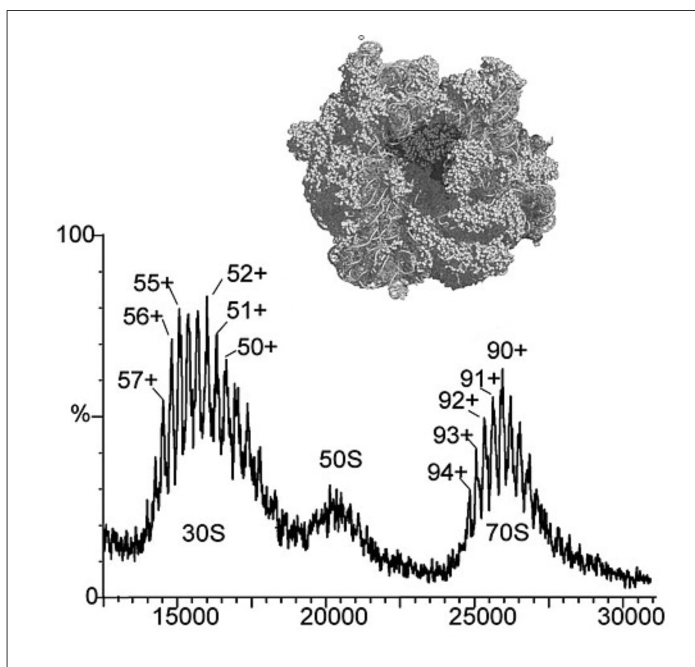

Fig. 4. Ribosome in the mass spectrometer

As a first example, Prof. Robinson presented the characterization of the trp RNA–binding attenuation protein (TRAP). It was possible to retain the undecameric, ring-like structure of TRAP in the gas-phase and even obtain the intact TRAP-trp-RNA complex where the trp-RNA is wrapped around the TRAP protein.[13] In a second example, Prof. Robinson showed that her group was able to establish the complete subunit architecture of the yeast exosome, the molecular machine responsible for degradation of RNA in yeast. This was achieved by generation of sub-complexes of the exosome in solution and by raising the internal energy to partially dissociate the exosome and its subcomplexes.[14] A third impressive example is the characterization of the human eIF3 complex involved in initiation of protein synthesis. The eIF3 complex has a molecular weight of about 800 kDa. Application of ion mobility spectrometry and controlled gas-solution phase dissociation allowed for identification of subcomplexes of eIF3 and it was possible to identify peripheral, loosely attached subunits.[15]

Carol Robinson

Structural Biology of RNA pol III Transcription

The final speaker of the Symposium was Dr. ***Christoph W. Müller*** (EMBL Heidelberg, Germany), presenting 'Structural Insight into RNA Polymerase III Transcription'. RNA pol III is a multiprotein assembly responsible for the transcription of tRNA and other small RNA encoding genes. With 17 subunits it is the largest of the three RNA polymerases present in eukaryotic cell nuclei, and has in yeast an aggregate molecular weight of ~700 kDa. The polymerase is recruited to tRNA promoters by concerted action of two further protein complexes, transcription factors TFIIIB and TFIIIC. Dr. Müller presented the X-ray structure of a TFIIIC subcomplex consisting of two subunits, τ60 and τ91. The complex features two WD40 seven-bladed propellers, one from each subunit, interacting in a hitherto unobserved, near perpendicular arrangement. The N-terminal end of τ91 showed DNA binding activity, and the C-terminal domain of τ60 was found to be arranged in a novel fold.[16]

Dr. Müller then presented the purification and structure solution at 17 Å by cryo-electron microscopy of the entire yeast RNA polymerase III holoenzyme. The initial structure was obtained by random conical tilt reconstruction to remove model bias. Fitting the structure of yeast RNA pol II and antibody labelling revealed the localization of RNA pol III specific subunits in the electron micrograph.[17] Dr. Müller concluded this final session of the Annual Symposium with a hypothetical model of a transcribing RNA polymerase III molecule based on his structural exploits (Fig. 5).

Christoph Müller and Carol Robinson

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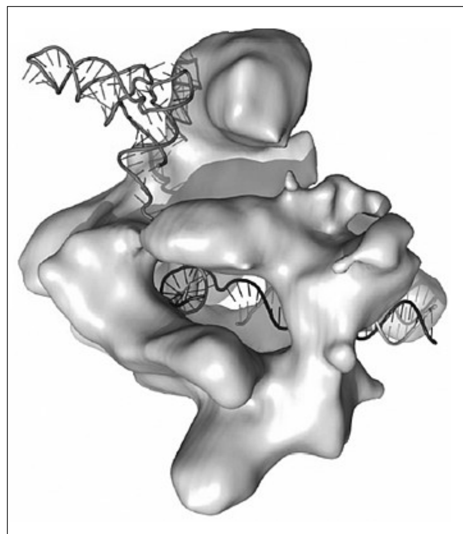

Fig. 5. Model of active RNA polymerase III based on electron microscopy data

Conclusions

The meeting was highly successful, with outstanding, internationally renowned speakers and a cutting-edge scientific program that allowed many stimulating discussions. It brought together scientists from chemistry, biology and other science backgrounds, which was beneficial and rewarding. Positively noted was the presence of numerous young scientists and students, who could experience a diverse mix of exciting topics in nucleic acid science presented by leaders of the field. The quality of the speakers and audience, as well as the generous support from academic institutions and industrial sponsors, testify for the continued appeal of the meetings organized by the Chemical Society Zürich. We are indeed looking forward to the CGZ Annual Symposium 2008.

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