The 2nd International Conference on Chemical and Structural Biology of Nucleic Acids and Proteins
&
The 2nd Symposium of Frontiers of Protein Sciences: from Structure to Function

Tsinghua University
Back Hall Auditorium, Main Building
May 19-22, 2012

Chairs: Yigong Shi & Wayne Hendrickson
Secretary General: Zhen Huang
Organizing Committee:
Yigong Shi, Wayne Hendrickson, Zhen Huang,
Lihe Zhang, Zhen Xi, Chengyu Jiang,
Haitao Li, and Ting Zhu.
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### Conference Agenda

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#### Sunday, May 20, 2012

**Session 1**

**Time:** 8:30 – 12:00  
**Place:** Back Hall Auditorium, Main Building  
**Chair:** Yigong Shi, Chuan He

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| 09:35 – 10:00 | Coffee break & Group Photo                                           |
| 10:00 – 10:30 | Wayne Hendrickson  
"Ligand Recognition and Plasticity in HIV Envelope Glycoprotein gp120" |
| 10:30 – 11:00 | Jian-Kang Zhu  
"Active DNA Demethylation in Arabidopsis" |
| 11:00 – 11:30 | Sunney Xie  
"Life at the Single Molecule Level" |
| 11:30 – 12:00 | Yigong Shi  
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#### Monday, May 21, 2012

**Session 2**

**Time:** 8:30 – 11:45  
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**Chair:** Wayne Hendrickson, Yijun Qi

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"Carba-LNA Modified Oligos (siRNA & Antisense) for Cellular mRNA Targeting" |
| 09:00 – 09:30 | Clemens Richert  
"Copying and Building with Synthetic DNA" |
| 09:30 – 10:00 | Jesper Wengel  
"UNA (unlocked nucleic acid) in RNA research" |
| 10:00 – 10:15 | Coffee break                                                         |
| 10:15 – 10:45 | Andreas Marx  
"Chemical Biology of DNA Replication" |
| 10:45 – 11:15 | Thomas Carell  
"Chemical Studies of Stem Cell Development" |
| 11:15 – 11:45 | Chengyuan Jiang  
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| 12:00 – 13:30 | Lunch & Poster Session                                               |
### Session 4

**Time:** 13:30 – 17:45  
**Place:** Back Hall Auditorium, Main Building  
**Chair:** Ruiming Xu, Wei Yang

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### Tuesday, May 22, 2012

**Beijing Tours**

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**The Origin of Life and the Emergence of Darwinian Evolution**

Jack W. Szostak  
Howard Hughes Medical Institute  
Massachusetts General Hospital  
Harvard Medical School  
2009 Nobel Laureate in Physiology or Medicine  
Email: szostak@molbio.mgh.harvard.edu

Dr. Szostak is an Investigator of the Howard Hughes Medical Institute, Professor of Genetics at Harvard Medical School, and the Alex Rich Distinguished Investigator in the Department of Molecular Biology at the Massachusetts General Hospital.

The earliest living cells must have had very simple structures in order to emerge spontaneously from the chemistry of the early earth. We are attempting to synthesize such simple artificial cells in order to discover plausible pathways for the transition from chemistry to biology. Very primitive cells (or protocells) may have consisted of a self-replicating nucleic acid genome, encapsulated by a self-replicating cell membrane. We have recently described simple and robust pathways for the coupled growth and division of primitive cell membranes composed of simple lipids that were likely have been available prebiotically. However, no process for the replication of a nucleic acid genome, independent of evolved enzymatic machinery, has yet been described. I will discuss our recent progress towards the realization of an efficient and accurate system for the chemical replication of RNA, as well as novel ways that ribozymes could potentially enhance RNA replication. Finally, I will describe ways in which ribozymes could alter protocell membrane composition so as to confer a strong selective advantage, thus leading to the emergence of Darwinian evolution through competition between protocells for a limiting resource.
Ligand Recognition and Plasticity in HIV Envelope Glycoprotein gp120

Wayne A. Hendrickson
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Crystallographic analyses of core portions of HIV gp120 glycoproteins showed how these viral envelope proteins bind to human CD4, the primary cellular receptor for HIV, and to a neutralizing antibody fragment, Fab 17b, whose binding epitope overlaps with that of HIV co-receptors CCR5 and CXCR4. HIV gp120 has a substantial cavity next to CD4 residue Phe43 at the focus of protein-protein interaction. Thermodynamic analyses indicated that gp120 undergoes remarkable conformational ordering when it binds CD4 or 17b. Subsequent structures of complexes with various antibody ligands show large conformational changes in gp120. We have derivatized a reactive F43C mutant variant of the D1D2 portion of CD4, replacing the Phe43 side chain with various chemical moieties and analyzing structure-activity relations for gp120 binding of resulting D1D2-F43C-X derivatives as reacted with a library of thiol-reactive compounds. HIV gp120 bound more tightly to some of these derivatives than to wild-type CD4. We determined crystal structures for five derivatized D1D2 proteins in complexes with core gp120 and Fab 17b. Chemical entities bound into the Phe43 cavity, which expanded in response to ligands. This plasticity in gp120 was associated with modified binding properties in cellular assays. We have also studied the complexes of several compounds derived from the gp120-ligand NBD-556 as bound directly to HIV gp120 molecules, both in complexes with antibody fragments and also without antibody ligands. Crystal structures of several such complexes reveal binding into the Phe43 cavity as for the D1D2-F43C-X derivatives. We have also performed extensive computational analyses of conformational plasticity in HIV gp120, including the use of our virtual-atom molecular mechanics (VAMM) force field to compute conformational transition pathways between various states of HIV gp120. These analyses help to characterize the conformational plasticity in the envelope glycoprotein that is crucial for its interaction with cellular receptors and for the mechanism for cell entry.

Active DNA demethylation in Arabidopsis

Jian-Kang Zhu
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Active DNA demethylation is involved in many vital developmental and physiological processes of plants and animals. In Arabidopsis, the ROS1/DME subfamily of DNA glycosylases function to promote DNA demethylation through a base excision-repair pathway. These specialized bifunctional DNA glycosylases remove the 5-methylcytosine base and then cleave the DNA backbone at the abasic site, resulting in a gap that is then filled with an unmethylated cytosine nucleotide by as yet unknown DNA polymerase and ligase enzymes. Recent evidence suggests that active DNA demethylation in mammalian cells is also mediated at least in part by a base excision repair pathway but much of the 5-methylcytosine first has to be oxidized and/or deaminated before a DNA glycosylase and other repair enzymes can act. I will present recent results on the identification and characterization of regulators and enzymatic components of the active DNA demethylation pathway in Arabidopsis.
Life at the Single Molecule Level

Sunney Xie
Mallinckrodt Professor of Chemistry and Chemical Biology,
Harvard University
Cheung Kong Visiting Professor,
College of Life Sciences Biodynamic Optical Imaging
Center, Peking University
E-mail: xie@chemistry.harvard.edu

Recent advances in single-molecule imaging in living cells allow quantitative and system-wide descriptions of gene expression and regulation with single molecule sensitivity. It was found that low probability stochastic of single molecules can have important biological consequences, such as the change of a cellular phenotype. This has everything to do with the fact that DNA are single molecules in individual cells. Meanwhile, recent advances in high throughput DNA sequencing have allowed sequencing the genome of a single human cell. The combination of single-molecule and single-cell imaging and sequencing offers exciting possibilities for biology.

Structural basis for the specific recognition of DNA by TAL effectors

Yigong Shi
Dong Deng, Chuangye Yan, Xiaojing Pan, Xinqi Gong, Magdy Mahfouz,
Jiawei Wang, Jian-Kang Zhu, Nieng Yan.
Tsinghua University, Beijing 100084, China
Email: shi-lab@tsinghua.edu.cn

TAL (transcription activator-like) effectors are the key virulence factors secreted by phytopathogenic bacteria Xanthomonas and injected into host cells through type III secretion system. They bind to target DNA via a central domain of tandem repeats. Each repeat recognizes a specific DNA basepair with two hypervariable residues (repeat variable diresidue or RVD) located at the 12th and 13th positions. Although the code of DNA recognition by RVDs was deciphered, the molecular basis for specific DNA recognition remained elusive. Here we present the crystal structure of an engineered TAL effector repeats, dHax3, in complex with DNA at 1.85 Å resolution. Each repeat is comprised of two helices connected by a short loop where RVD locates. The 11.5 repeats are organized into a super-helical structure that encompasses the major groove of the DNA double helix with RVDs directly contacting the bases. Structural comparison with DNA-free dHax3, whose structure was determined at 2.4 Å resolution, revealed molecular basis for the structural malleability of the repeats.
RNA architecture can be viewed as the hierarchical assembly of preformed doublestranded helices defined by Watson-Crick base pairs and RNA modules maintained by non-Watson-Crick base pairs. RNA modules are recurrent ensemble of ordered non-Watson-Crick base pairs. Such RNA modules constitute a signal for detecting noncoding RNAs with specific biological functions. It is, therefore, important to be able to recognize such genomic elements within genomes. Through systematic comparisons between homologous sequences and X-ray structures, followed by automatic clustering, the whole range of sequence diversity in recurrent RNA modules has been characterized. These data permitted the construction of a computational pipeline for identifying known 3D structural modules in single and multiple RNA sequences in the absence of any other information. Any module can in principle be searched, but four can be searched automatically: the G-bulged loop, the Kink-turn, the C-loop and the tandem GA loop. The present pipeline can be used for RNA 2D structure refinement, 3D model assembly, and for searching and annotating structured RNAs in genomic data.

Following the recent dramatic advances in tools aimed at RNA 3D modeling, a first, collective, blind experiment in RNA three-dimensional structure prediction has been performed. The goals are to assess the leading edge of RNA structure prediction techniques, compare existing methods and tools, and evaluate their relative strengths, weaknesses, and limitations in terms of sequence length and structural complexity. The results should give potential users insight into the suitability of available methods for different applications and facilitate efforts in the RNA structure prediction community in their efforts to improve their tools.

Five structurally distinct classes of catalytic RNAs that self-cleave through an internal transesterification reaction have been discovered in nature: the glmS, hairpin, hammerhead, HDV, and VS ribozymes. The hammerhead and HDV ribozymes have been found in all phyla, while the glmS ribozyme-riboswitch is prevalent in Gram-positive bacteria. Among these five classes of ribozymes, the glmS is unique in requiring a small-molecule coenzyme, glucosamine-6-phosphate (GlcN6P) for catalysis. While this requirement is consistent with the role of the glmS ribozyme-riboswitch in controlling expression of the protein enzyme GlcN6P synthetase (in whose mRNA the riboswitch resides), it contrasts with the ability of the other four classes of self-cleaving ribozymes efficiently to catalyze the same reaction without any cofactors (except Mg²⁺ in the case of the HDV ribozyme). Moreover, previous in vitro selection (SELEX) experiments have shown that coenzyme-independent self-cleaving ribozymes are widespread in RNA sequence space. Did the glmS ribozyme evolve from an ancestral GlcN6P-independent self-cleaving ribozyme of similar overall structure? We experimentally addressed this question by subjecting a pool of mutagenized glmS ribozyme RNA pools to in vitro selection for specific self-cleavage in the absence of any small molecule cofactors. Analysis of sequences obtained after ten rounds of SELEX indicates that just three point mutations suffice to confer GlcN6P-independent cleavage activity to the glmS ribozyme. We find that GlcN6P, various organic buffers, or cobalt (III) hexamine do not support self-cleavage of this triple mutant, which appears to have a specific requirement for Mg²⁺ for optimal catalytic activity. Crystallographic analysis demonstrates that the triple-mutant RNA adopts the same overall fold as the wild-type glmS ribozyme, and that the mutations abrogate the GlcN6P binding pocket. Kinetic analysis of RNAs containing the three point mutations in various combinations shows that conversion of any of the three nucleotides from the mutant ribozyme to wild-type results in the mutant ribozyme losing its ligand independent self-cleave activity, while conversion of one of the three nucleotides to wild-type results in recovery of substantial GlcN6P-dependent activity. This study illustrates how RNAs of distinctly different biochemical functionality (GlcN6P-dependent and independent self-cleave, in this case) populate adjacent portions of sequence and structure space. This property might provide the basis for the facile evolution of RNAs with novel activities. On the other hand, the susceptibility of a particular RNA activity to deleterious point mutations suggests that RNA might have difficulty in developing resistance to drugs while retaining a phenotypically important biochemical activity.
The k-turn : a key architectural element in RNA

David M.J. Lilley
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The kink-turn (k-turn) is a widespread and important element in the architecture of RNA molecules. It generates a local kink in an RNA helix with an included angle of 60°, thus mediating long-range interactions in folded RNA. k-turns occur in both ribosomal subunits, in box C/D and H/ACA snoRNA, the U4 snRNA and some riboswitches. They are therefore important in translation, RNA modification and splicing, and control of gene expression.

In the absence of metal ions, the k-turn adopts a less-tightly kinked structure, but can be induced to fold into its kinked conformation by one or more of three processes. (1) By the addition of metal ions in a two-state folding process. (2) The binding of specific proteins can induce the folding of k-turns. This can occur with very high affinity; for example A. fulgidus L7Ae induces the folding of Kt-7 with an apparent affinity of $K_d = 10$ pM. Other ribosomal proteins including L24, S17 and S11 also induce the formation of k-turn structure. We have analyzed the process of protein-induced folding of the k-turn using single-molecule experiments. (3) Tertiary contacts in species such as the SAM-I riboswitch can stabilize the folded form of the k-turn, and indeed stabilize k-turns that are unable to fold by addition of metal ions. Thus potentially k-turns can play a key role in RNA folding in a delicate interplay involving sequence variation, protein binding and tertiary contacts.

We have dissected the role of hydrogen bonding in stabilizing the folded conformation of the k-turn in detail. We show that the k-turns exist in two structural classes that differ in key hydrogen bonding patterns. Moreover, the same sequence can adopt either structure depending upon its environment. We have determined the crystal structure of Kt-7 in a variety of situations, showing the variation in geometry as a function of protein binding and tertiary contacts.

We have created a web-based database of k-turn structures, which can be found at http://www.dundee.ac.uk/biocentre/nasg/ktturn/.

DNA translesion synthesis: from chemistry to cancer

Wei Yang

Wei Yang 1, Ye Zhao 1,2, Teruya Nakamura 1,3, Christian Biertuempfel 1,4, Fumio Hanaoka 5
1 LMB, NIDDK, NIH, 2 Zhejiang University (China), 3 Kumamoto University (Japan), 4 Max Planck Institute of Biochemistry (Germany), 5 Gakushuin University (Japan)
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Human DNA pol h is essential for UV survival. Deficiency of DNA pol h causes the variant form of Xeroderma Pigmentosum (XPV), characterized by sunlight-induced pigmentation changes and a highly elevated incidence of skin malignancies. Interestingly, DNA pol h is a “double agent” in cancer biology. It counteracts the DNA-damaging effect of cisplatin in anti-cancer treatment by carrying out accurate translesion synthesis opposite intra-strand cisplatin crosslinked guanines. In this talk, I will compare the mechanism of accommodation of UV- versus platinum-induced double bases by human DNA pol h and the differences between the human and yeast homologs. Finally, I will present our most recent results on dissecting the chemical steps of DNA synthesis.
Creating Novel Base Pairs for Nucleic Acid Recognition and Evolution

Zhen Huang
Huiyan Sun, Jia Sheng, Abdalla Hassan, Wen Zhang, Sibo Jiang, Jianhua Gan, Zhen Huang*
Department of Chemistry & Department of Biology, Georgia State University, Atlanta, GA, USA,*
*Email: Huang@gsu.edu

Specificity of nucleobase pairing provides essential foundation for genetic information storage, replication, transcription and translation in all living organisms. However, the wobble base pairs, where U in RNA (or T in DNA) pairs with G instead of A, compromise the high specificity of the base pairing. To increase U/A pairing specificity, we have hypothesized to discriminate against U/G wobble pair by tailoring the steric and electronic effects at the 2-exo position of uridine and replacing the 2-exo oxygen with a selenium atom. We report here the first synthesis of novel 2-Se-uridine RNAs. Our biophysical and structural studies reveal that this single atom replacement can indeed create a novel U/A base pair with higher specificity than the natural one, while maintaining the native structure. This oxygen replacement with selenium (Ref. 1-8) offers a unique chemical strategy to enhance base pairing specificity and preserve genetic information at the RNA level. This work is supported by NIH (R01GM095881 and GM095086) and NSF (MCB-0824837 and CHE-0730235).

Fig. 1 Selenium-modified T/A (or U/A) and T/G (or U/G) wobble pairs

References:

Reversible Epigenetic Methyllations of DNA and RNA in Mammalian Cells

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Reversible chemical modifications on nucleic acids and proteins determine cell fates. The five bases that comprise nucleic acids - adenine, guanine, cytosine, thymine, and uracil - can be chemically and enzymatically modified. These chemical events can have significant biological consequences, particularly for gene expression. I will present chemical strategies we have developed to enrich, sequence, and study novel nucleic acid modifications that include 5-hydroxymethylcytosine and its further oxidized forms in mammalian genome. Implications for demethylation will be discussed. Besides the well-known DNA methylation, mammalian messenger RNAs (mRNA) are ubiquitously methylated on selected adenosines. We have recently shown that the human fat mass and obesity-associated protein FTO is the first mRNA demethylase discovered. I will present our recent results that reveal additional reversible methylation of mammalian messenger RNA catalyzed by these RNA demethylases. Based on these discoveries we propose a new mode of biological regulation that depends on reversible RNA modification, for which we termed “RNA Epigenetics”.

Specificity of nucleobase pairing provides essential foundation for genetic information storage, replication, transcription and translation in all living organisms. However, the wobble base pairs, where U in RNA (or T in DNA) pairs with G instead of A, compromise the high specificity of the base pairing. To increase U/A pairing specificity, we have hypothesized to discriminate against U/G wobble pair by tailoring the steric and electronic effects at the 2-exo position of uridine and replacing the 2-exo oxygen with a selenium atom. We report here the first synthesis of novel 2-Se-uridine RNAs. Our biophysical and structural studies reveal that this single atom replacement can indeed create a novel U/A base pair with higher specificity than the natural one, while maintaining the native structure. This oxygen replacement with selenium (Ref. 1-8) offers a unique chemical strategy to enhance base pairing specificity and preserve genetic information at the RNA level. This work is supported by NIH (R01GM095881 and GM095086) and NSF (MCB-0824837 and CHE-0730235).
DNA Oxidation towards Totipotency in Mammalian Development

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Fragmentation of chromosomal DNA is a critical step in apoptosis that prevents a cell from transcribing and replicating its genes and thus facilitates the cell killing process. Defects in this process can cause various pathological conditions, including autoimmune disorders and cancer. Using a combination of genetic, biochemical and functional genomic approaches, we have identified ten apoptotic nucleases and several non-nuclease factors involved in regulating and executing apoptotic chromosome fragmentation in C. elegans. They act in a sequential and CED-3 caspase-dependent manner to promote stepwise fragmentation and degradation of chromosomes. The process is initiated by a novel CED-3-mediated conversion of the dicer ribonuclease (RNase) into a deoxyribonuclease (DNase), which makes the first cuts on chromosomes. In parallel, a mitochondrial nuclease CPS-6 (a homolog of human endonuclease G) and its activator WAH-1 (a homolog of human apoptosis-inducing factor) are released from mitochondria and translocated to the nucleus, where they interact and cooperate with other cell death nucleases to turn the initial cuts by dicer into double-strand chromosome DNA breaks, leading to fragmentation and degradation of chromosomes. Unexpectedly, defects in chromosome fragmentation also compromise clearance of apoptotic cells, suggesting that the apoptotic chromosome fragmentation process and the cell corpse engulfment process are intrinsically connected. Indeed, from a CED-3 protease suppressor screen, we have identified two genes, cps-13 and cps-14, that affect both apoptotic chromosome fragmentation and cell corpse clearance. Further molecular genetic and biochemical analyses of these cell death nucleases and non-nuclease regulators in C. elegans will help elucidate how the apoptotic chromosome fragmentation process and the cell corpse engulfment process are regulated and coordinated to promote cell death.

Dicing up chromosomes during apoptosis

Ding Xue

Akihisa Nakagawa,1 Xiao Ge,2 Xiang Zhao,2 Yong Shi,1 Jay Parrish,1 and Ding Xue2,2
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Sperm and eggs carry distinctive epigenetic modifications that are adjusted by reprogramming following fertilization. The paternal genome undergoes active DNA demethylation before the first mitotic division. The biological significance and mechanisms of paternal epigenome remodeling are unclear. We find that, within mouse zygotes, oxidation occurs in the paternal genome, changing 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC). In Tet3 oxidase-deficient zygotes derived from conditional knockout mice, the conversion of 5mC into 5hmC fails to occur. Thus, the loss of 5mC in the paternal genome in zygotes is caused by its conversion to 5hmC mediated by Tet3. Deficiency of Tet3 also impedes demethylation at the paternal copy of genes such as Oct4 and Nanog and delays the subsequent reactivation of Oct4 in early embryos. Heterozygous mutant embryos lacking maternal Tet3 suffer increased developmental failures, with female mice depleted of Tet3 in the germ line displaying severely reduced fecundity. Importantly, oocytes lacking Tet3 also show impaired reprogramming of injected somatic cell nuclei. We conclude that Tet-mediated oxidative DNA demethylation is essential for epigenetic reprogramming in the early embryo following natural fertilization, as well as for the reprogramming of somatic cell nuclei during animal cloning.
Carba-LNA modified Oligos (siRNA & Antisense) for Cellular mRNA Targeting

Jyoti Chattopadhyaya
Program of Chemical Biology (ICM), Box 581, Biomedical Centre, Uppsala University, SE-75123 Uppsala, Sweden
Email: jyoti@boc.uu.se; Website: www.boc.uu.se

Fine tuning of the electrostatic properties around the internucleotidic phosphate can be achieved by incorporations of lipophilic vs hydrophobic substituents on the Carba-LNAs and –ENAs leading to significant modulation of the antisense and small interfering RNA (siRNA) properties, such as target affinity, nuclease resistance and RNase H or the ago protein elicitation. This study, with synthetic chemistry, enzymology and NMR structure, gives an insight on the importance of chemical characters of the substituent-type in the carbocyclic moiety of carba-LNA and carba-ENA in the minor groove for the design of the RNA targeted therapeutics.

Upon screening of 52 modified antisense oligonucleotides, containing 13 differently functionalized carba-LNA/ENA derivatives, two excellent modifications have been found, which facilitate excellent target RNA affinity, nuclease resistance and RNase H activity, and they are deemed to be excellent candidates as potential antisense and siRNA therapeutic agents against target mRNA.

This study finally shows how the appropriate RNA target selection in the HIV genome and their specific inhibition by the siRNA approach by the choice of appropriate chemistry can also successfully modulate the expression and inhibition of HIV-specific proteins. In summary, we will discuss here the key role of innovative chemistry responsible in steering of the biological function (Chemistry-Biology interplay).

Speaker thanks Swedish Research Council (VR) and EU Framework programs for funding of this research, and also thanks all cited authors for their excellent contributions

Some Current Relevant References (For full list, see: www.boc.uu.se)


Nature evolved DNA to store genetic information. But, DNA may also be used to build designed nanoscale objects.\(^1\) We have recently shown that branched DNA hybrids, whose oligonucleotide arms are linked by rigid organic cores, assemble at a chain length much shorter than that required for duplex formation between linear DNA strands.\(^2\) Dimers of strongly pairing nucleotides (CG) or tetramers of weakly pairing nucleotides (TA) were found to produce solids from micromolar solutions in aqueous buffer upon addition of divalent cations. Soon afterwards, Seeman and coworkers reported designed DNA crystals, assembled from rigid folding motifs with dimer sticky ends.\(^3\) To bring branched oligonucleotides into the realm of materials that may be used for practical applications, a solution-phase synthesis of DNA hybrids with CG zipper arms was developed, together with protocols for the assembly of the resulting DNA hybrids from micromolar aqueous solutions.\(^4\) By varying the branching geometry, rigidity, and charge of cores, we were able to identify hybrid structures that assemble into macroscopic materials at temperatures as high as 95 °C.\(^5\)

RNA can act as genetic material and as catalyst. Life most probably evolved from simple self-replicating RNA systems.\(^6\) It is interesting to ask how the first template-directed copying of RNA sequences occurred, in the absence of polymerases.\(^7\) We have studied primer extensions on weakly pairing sequences that were deemed blocks for enzyme-free replication.\(^8\) Recently, we were able to show that efficient copying of mixed sequences, containing all four nucleobases, occurs on immobilized templates.\(^9\)

References
UNA (unlocked nucleic acid) in RNA research

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UNA (unlocked nucleic acid; 2',3'-seco-RNA) is an acyclic form of RNA that reduces the thermal stability of nucleic acid duplexes and therefore in many ways can be considered the antipode of LNA (locked nucleic acids) within the arsenal of synthetic RNA mimics. In the lecture it will be discussed how UNA modifications of oligonucleotides, siRNA constructs, miRNAs and aptamers can lead to novel and appealing properties. For a recent review, see M. A. Campbell and J. Wengel, Chem. Soc. Rev. 2011, 40, 5680.

Chemical Biology of DNA Replication

Andreas Marx
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DNA is the storage of genetic information in Nature. Transmission of the genetic information from the parental DNA strand to the offspring is crucial for the survival of any living species. The entire DNA synthesis in DNA replication is catalyzed by DNA polymerases and depends on their ability to select the canonical nucleotide from a pool of structurally similar building blocks. Besides the crucial biological role of DNA polymerases, these enzymes are the workhorses in numerous important molecular biological core technologies such as the ubiquitous polymerase chain reaction (PCR), cDNA cloning, genome sequencing, and nucleic acids based diagnostics.

We will report results on insights into how DNA polymerases faithfully recognize their template, how chemically modified nucleotides with potential in biotechnological applications are processed, and finally, new approaches to study the orchestration of human DNA polymerases by mimicry of posttranslational protein modification.
Chemical Studies of Stem Cell Development
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In the lecture I will discuss the latest results about the function and distribution of the new nucleobases 5-hydroxymethylcytosine (hmC),[1] 5-formylcytosine (fC),[2] and 5-carboxylcytosine (caC).[3] These nucleobases play an important role in epigenetic reprogramming of stem cells and some of these bases are also detected at relatively high levels in brain tissues. I will discuss new synthetic routes to these compounds and to the corresponding phosphoramidites using modern metal organic chemistry and I will discuss how chemistry leads to new insights into the biology of stem cell development processes.[4]

In particularly mass spectrometry in conjunction with the availability of the synthetic material in isotopically labeled form enables us today the investigation of the distribution of these novel bases in various tissues and during stem cell development.[5] I will show that the base formylcytosine, which was discovered by us,[2] is present in stem cells and that the distribution varies with time in a wave like fashion. I will then discuss in detail the distribution of carbonylcytosine in somatic tissues and in stem cells and will show the discrepancies existing in the literature about this base. New information derived from a detailed mass spectrometric analysis will be provided.

Autophagy Inhibitors: Potential Chemical Drugs for Treating Acute Lung Injury Caused by Avian Influenza A H5N1 Infection
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The threat of a new influenza pandemic has been impending since 1997, when a highly pathogenic strain of avian influenza A H5N1 infected humans in Hong Kong and spread across Asia, continuing to infect both poultry and people. The human mortality rate of H5N1 infection is about 60 percent, while that of seasonal H1N1 infection is less than 0.1 percent. The high mortality rate from H5N1 infection is predominantly due to respiratory failure caused by acute lung injury. It remains poorly understood how the viral infection of lung cells contributes to this disease pathology. Here, we show accumulation of autophagosomes in electron microscope images of H5N1-infected lung tissues of a human cadaver and mouse and from H5N1-infected A549 human epithelial lung cells. We also show that H5N1 induces alveolar epithelial autophagic cell death through the Akt-TSC2-mTOR pathway, while seasonal H1N1 does not. Reagents blocking this virus-induced autophagic signaling pathway strikingly increased the animal survival rate when applied prophylactically and significantly ameliorated the acute lung injury and mortality caused by H5N1 infection in animals when used therapeutically. We conclude that alveolar epithelial autophagic cell death likely plays a crucial role in the high mortality rate of H5N1 infection, and we propose that autophagy-blocking agents may be useful as prophylactics or therapeutics against a potential pandemic of human H5N1 influenza.

Both theoretical and experimental work demonstrated that a single RNA sequence can assume multiple, distinctly folded structures with different functions. These structures, or more precisely conformations, are different structural folds that can be reversibly generated through unfolding/refolding. For example, the same RNA sequence can adopt a fold that catalyzes RNA cleavage or a different fold that catalyzes RNA ligation. On binding of small metabolites, riboswitches can switch their conformations and consequently functions. However, here we show that a single RNA sequence assumes two structures with two different functions, both of which are required to work together in order to competitively inhibit the GluR2 AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor with an IC50 value of ~30 nM. Yet, the two structures, once formed during in vitro transcription, are not interconvertible through unfolding or denaturation/refolding. The sequence we present corresponds to an aptamer, which was identified from systematic evolution of ligands by exponential enrichment (SELEX) against the GluR2 receptor from an RNA library. GluR2 is an AMPA receptor subunit of the glutamate ion channel family, and plays a key role in brain development and function. Excessive activity of the GluR2 AMPA receptors has been implicated in a number of neurological disorders, and therefore inhibitors, such as RNA aptamers, might be useful as pharmacological tools. To understand the RNA structure-function relationship, we have carried out, and describe here, a series of experiments, including in-line probing, chemical labeling and enzymatic digestion, to demonstrate that these RNA species have the same sequence, but are structurally and functionally distinct. Our results suggest more broadly that natural RNA molecules that show structural dissimilarities with different functions can nevertheless share a common ancestry and bear the same evolution memory.

RNA interference (RNAi) represents a powerful molecular strategy for inhibition of gene expression. This process can be induced by 21–23 nt double-stranded RNAs (dsRNAs), known as small interfering RNAs (siRNAs). These oligoribonucleotides (formed by a sense and an antisense strand) are recognized by the RNA-induced silencing complex (RISC), a protein complex located in the cytoplasm.

By the strategy of isonucleoside modification, it was found that sense strand of siRNA was more tolerant to be modified, and obviously affected the silencing activity initiated by itself. While only 3'-end of antisense strand was tolerant to be modified.

To increase cell permeability of siRNA, peptide(Cell penetrating peptides, LALLAK)-siRNA conjugates have been obtained by a solid-phase chemistry strategy, which peptide derivative CPG(Controlled Pore Glass) and then oligonucleotide synthesis followed. The preliminary silencing result indicated that 3'-sense strand conjugates kept good gene silencing activity, while the 3'-antisense strand conjugates decreased slightly. This work was supported by the NSFC (20932001), the Ministry of Science and Technology of China (2009ZX09503).

4. L. Pei, LJ Xie, Q Li, XM Ling*, Z Guan, ZJ Yang*, Studies on the adenosine deaminase-catalyzed conversion of adenosine and nucleoside prodrugs by different capillary electrophoresis modes, Anal. Biochem., 2011, 414, 131-137
RNA silencing is a conserved mechanism for gene regulation in most eukaryotes, in which small noncoding RNAs (e.g., siRNAs, miRNAs and piRNAs) are central components to silence the target genes through sequence specific base-pairing. In particular, miRNAs play essential roles in numerous developmental processes in animals and plants, such as organ development and stem cell maintenance. Aberrant reduction or elevation in the level of these small noncoding RNAs are associated with many developmental and physiological defects. So the steady-state levels of small noncoding RNAs are precisely regulated through modulating their biogenesis and turnover. The 3'-end modification of small noncoding RNA is a common mechanism to modulate their stabilities. Except miRNAs in animals, most small noncoding RNAs are usually 3'-end 2'-O-methylated by methyltransferase HEN1, which increases their stabilities. The 3'-end nucleotide addition of unmethylated small noncoding RNAs by various nucleotidyl transferases, predominantly uridylation and adenylation, influence their stabilities and functions. 3'-5' exoribonuclease regulate the steady-state level of small noncoding RNAs through distinct mechanisms. In Drosophila, many miRNAs undergo 3' trimming by the exoribonuclease Nibbler, which enhances their activities. In Arabidopsis, a family of exoribonucleases encoded by SMALL RNA DEGRADING NUCLEASE (SDN) genes in Arabidopsis, specifically degrades mature single-stranded (ss) miRNAs. We are interested in studying the molecular bases of these mechanisms involved in small noncoding RNA metabolism, and our recent works will be presented in the talk.

Small RNAs in Plants: More Surprises

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Small RNAs are important components in the eukaryotic gene regulatory networks. Plants have evolved a complex system of small RNAs, among which the predominant species are microRNAs (miRNAs) and heterochromatic siRNAs (hc-siRNAs). miRNAs mediate post-transcriptional gene regulation through target mRNA cleavage or translational repression, whereas hc-siRNAs direct de novo DNA methylation at homologous loci through a pathway known as RNA-directed DNA methylation (RdDM). Recently, my lab has discovered a novel class of small RNAs that are induced by DNA double-strand breaks (DSBs) in Arabidopsis and humans. These DSB-induced small RNAs (diRNAs) play important roles in DSB repair. In my talk, I will present our recent findings with regard to the mechanisms of hc-siRNA-directed DNA methylation and diRNA-mediated DSB repair.
Epigenetic control of gene expression involves alterations of higher order chromatin structure, which is governed by a number of factors including covalent modifications of histones. A key area remains poorly understood is the molecular mechanisms by which the activities of histone modification enzymes are regulated. The budding yeast NAD-dependent histone deacetylase Sir2 is crucial for epigenetic silencing, and its enzymatic activity is stimulated by the binding of Sir4. I will present the structure of the Sir2-Sir4 complex, and our analysis of the molecular mechanism of allosteric stimulation of Sir2 activity.

The dosage compensation complex (DCC) binds to single X chromosomes in Drosophila males and increases the transcription level of X-linked genes by approximately twofold to match that from two female X chromosomes. The DCC is a RNA-protein complex that contains at least five proteins male-specific lethal 1 (MSL1), MSL2, MSL3, the acetyltransferase MOF and maleless, and two non-coding RNAs roX1 and roX2. The transcriptional activation is caused, at least in part, by the MOF-mediated acetylation of histone H4 lysine 16 and enhanced transcriptional elongation. The DCC is specifically localized on the X chromosome in male flies, but the mechanism remains poorly understood. According to a prevalent model, the DCC first binds to a limited number of high-affinity sites and then spreads in cis to flanking active genes. The initial X-targeting step likely involves specific recognition of a short DNA sequence motif enriched in high-affinity sites. The CXC domain of MSL2 is required for faithfully targeting the DCC to the X chromosome. We have determined the structure of CXC domain of MSL2 and analyzed its DNA-binding property.
Crystal structure of the N-terminal domain of ZAP

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Application of NMR-based drug screening techniques to study the chemical replication as a model for the origin of life

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Type I interferon (IFN) can block virus replication by stimulating the expression of various antiviral genes. Zinc-finger antiviral protein (ZAP) is one of the interferon-stimulated genes that specifically inhibits the replication of certain viruses such as HIV-1 and Ebola virus by targeting viral mRNA for degradation. No common RNA motif has been identified that can be recognized by ZAP. The only feature of these ZAP targets is over 500nt long. How ZAP recognizes its target RNA was unclear. We determined the crystal structure of the major functional domain of rat ZAP (N-ZAP225). The overall structure of N-ZAP225 resemble a tractor in which the four zinc-finger motifs of this protein locate at the bottom as the four wheel of a tractor. Structural and functional analyses identified multiple positively-charged residues and two putative RNA binding cavities forming a large putative RNA binding cleft. ZAP molecules interact with each other to form a dimer in the structure and in our in vitro and in vivo assays. Accordingly, ZAP-target RNA molecule Na also contains two ZAP-binding modules. Based on these results, we proposed a model for the functional mechanism of ZAP to recognize its target RNA.

Non-enzymatic template-directed polymerization of 5’-phosphate chemically activated mono-ribonucleotides (activated monomers) has been studied as a model for chemical RNA replication during the origin of life. This chemical polymerization proceeds initially by the binding of the activated monomers to a complementary single stranded template via non-covalent interactions including base pairing and stacking. A phosphoryl transfer reaction between adjacent bound monomers subsequently forms a covalently linked sugar-phosphate backbone of the new product strand complementary to template strand. As a result, the genetic information transfer could be eventually achieved through this spontaneous chemical template copying.

Due to the templated nature of this reaction, the efficiency of polymerization depends not only on the reactivity of the activated monomers, but also on how tightly the monomers associate with the template and on the conformation adopted by the template-bound monomers. Our results of NMR waterLOGSY and STD experiments confirm that a positively cooperative binding mode is adopted when monomers bind to complementary ss-template with multiple adjacent binding sites. This binding cooperativity allows primer-independent chemical template copying to proceed with simultaneous initiation at multiple sites (concerted-like reaction) as opposed to the stepwise reaction pattern. In addition, the cooperative binding also enhances fidelity of chemical replication in which the inherently dominant occurrences of Watson-Crick over mismatched base pairings are adopted when monomers bind to the template-bound monomers. Our results provide a structural correlation to observations that activated ribonucleotides are superior to activated deoxyribonucleotides, and that RNA templates are superior to DNA templates in template-directed non-enzymatic primer-extension reactions.

Furthermore, we used Transferred NOESY experiments, which can be used to specifically detect the bound conformation of small molecule ligands with relatively weak affinity to receptors, to study the interactions between activated monomers and single-stranded oligonucleotide templates. We found that the sugar pucker of activated ribonucleotides switches from C2’-endo in the free state to C3’-endo upon binding to an RNA template. This switch occurs only on RNA and not on DNA templates. Our results provide a structural correlation to observations that activated ribonucleotides are superior to activated deoxyribonucleotides, and that RNA templates are superior to DNA templates in template-directed non-enzymatic primer-extension reactions.

The NMR experiments mentioned above have been widely used in the field of drug discovery. These techniques are able to offer valuable binding mode information such as epitopic mapping and the conformation of a ligand in bound state. Especially, hits can be directly identified from a mixture of several test compounds simultaneously, which significantly increases screening throughput.
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