MEETING REVIEW

Viewing the ribosome and visiting the nucleolus at Lake Tahoe

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A meeting “Ribosome Biogenesis and Nucleolar Function” was held at Lake Tahoe, California, August 17–21, 2000, ably organized by Lasse Lindahl and Janice Zengel (University of Maryland, Baltimore County), together with Maurille Fournier (University of Massachusetts), Craig Pikaard (Washington University), and John Woolford (Carnegie-Mellon University). The meeting was noteworthy in several respects. First, it occurred within only a few days (on either side) of the publication of breathtaking 2.4–3.3 Å structures of the large and small ribosomal subunits. Second, it addressed how this machine is built in the cell to a greater extent than most previous ribosome meetings. Third, the meeting congratulated Masayasu Nomura for his important, and still continuing, contributions to the ribosome field.

After a memorial tribute in the opening session to the late Rudi Planta, the meeting’s organization unfolded with Aristotelian logic, starting with rRNA gene expression, in which welcome biochemical and genetic progress on the ribosomal gene transcription machinery was presented. With the possible exception of pol I itself, all the rDNA transcription components are now pure proteins and we await the next (crystallographic) steps.

A longstanding issue has been whether nucleoli are simply the cytological manifestation of rDNA transcription and nascent ribosome assembly or are first assembled by other factors. Human acrocentric chromosomes display a remarkable propensity to associate with ribosomal RNA genes—the nucleolar organizers, which can end up constituting 25–33% of the entire short arm of chromosomes 13, 14, 15, 21, and 22. These arms expectedly nucleate prenucleoli in telo-

phase, which then fuse to form the larger nucleoli that typify the interphase landscape. Surprisingly, this essential role of the rDNA in nucleolar assembly does not require its transcriptional activity, as revealed by a clever exploitation of species-specific rDNA transcription in mouse/human cell hybrids (B. McStay, University of Dundee). Thus, it appears that the initial signal for assembling a nucleolus is something more primordial than rDNA transcription itself.

A look into intriguing relationships between the nucleolus and rDNA expression on the one hand and broader aspects of cell regulation on the other was offered by Masayasu Nomura (University of California, Irvine). The 35S rRNA genes on chromosome XII in *Saccharomyces cerevisiae* have an enhancer at the end of each module in the repeated array, initially identified by Jonathan Warner’s lab and further characterized by Shirleen Roeder and colleagues in connection with their work on *HOT1*-dependent stimulation of recombination. This enhancer’s role was defined using ectopic reporter genes linked to the pol I promoter. Yet, Nomura reported that deletion strains of this putative enhancer grow at wild-type rates and display normal rRNA synthesis. In addition, he showed that another mutation, *lob1*, abolishes enhancer-dependent transcription at an ectopic pol I promoter in a *HOT1* system, without any effect on chromosomal rDNA transcription. The *LOB1* gene encodes a hot-spot related protein, which plays an essential role in recombination within the rDNA repeats. Nomura’s results imply that the *LOB1* gene product together with the enhancer plays a role in recruiting pol I and related transcription machinery to ectopic rDNA in the previous studies. He suggests that the *LOB1* gene product stimulates an interaction between rDNA repeats through the enhancer, explaining its role in recombination as well as its requirement for transcription from ectopic pol I promoters. These results emphasize that the nucleolus

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is functionally compartmentalized from the extra-nucleolar regions and that pol I and its transcription factors are not readily available for genes outside the nucleolus.

Pondering the numerous processing enzymes that operate on the several different classes of eukaryotic RNA, one can either be confused or search for underlying meaning. The latter disposition was exemplified in a talk that noted, as its point of departure, the broad RNA species substrate spectrum of putative "rRNA processing enzymes," that is, there is no known processing enzyme specific for pre-rRNA (D. Tollervey, University of Edinburgh). Thus, yeast Rntlp, a component of the rRNA processing enzyme RNase MRP, also processes the precursors of the spliceosomal U1, U2, U4, and U5 snRNAs, the monocistronic U3 snoRNA, and several polycistronic snoRNAs. Ratlp, a pre-rRNA 5' exonuclease also processes pre-snoRNAs and participates in nuclear pre-mRNA degradation. The cytoplasmic rRNA 5' end processing exonuclease Xrnlp degrades mRNAs. The 5' → 3' "exosome," initially described as a rRNA processing machine, now has been found to also process pre-snRNAs and pre-snoRNAs, and also to degrade both nuclear pre-mRNAs and cytoplasmic mRNAs. The Rex1-3p gene product, initially thought to be solely a pre-rRNA 3' processing enzyme, also processes pre-snRNAs and RNase P RNA. Finally, the Lsm gene product, implicated in both endo- and exonucleolytic processing at several steps of pre-rRNA processing, is also involved in pre-snRNA, pre-snoRNA, and pre-tRNA processing and cytoplasmic mRNA turnover. From all these observations, Tollervey courageously proposed the existence of a single RNA processing system. Notwithstanding the spliceosome (which conducts a different chemistry of phosphodiester attack), this idea has no compelling counterarguments at present as far as this observer is aware.

The small nucleolar RNAs that serve as guides for ribosomal RNA ribose 2'-O-methylation and pseudouridine formation were the subject of several presentations that reported steady progress. One study identified four novel brain-specific box C/D snoRNAs in human and rodents (J. Cavaillé from the group of J.-P. Bachellerie, Centre National de la Recherche Scientifique (CNRS) and Université Paul Sabatier, Toulouse). The genes encoding three of these map to a region of human chromosome 15 encompassing a locus involved in Prader-Willi syndrome (PWS), a neurological disorder related to a paternal gene expression deficiency. These three snoRNAs were absent from brain cortical tissue of an autopsied PWS patient and were also non-expressed in brain tissue in a mouse PWS disease model. The fourth brain-specific snoRNA revealed in this study has, surprisingly, no complementarity to rRNA or any of the spliceosomal RNAs. This latter snoRNA comes from a brain-specific repetitive DNA sequence transcript that lacks any mRNA coding elements.

As mentioned, a major focus of the meeting was ribosome biogenesis. The pathway of rRNA processing has of course been worked out in some detail, but the parallel schedule of ribosome assembly has remained less well defined, even though nascent ribosomes were first isolated over 30 years ago. Welcome progress abounded at the meeting. Yeast nucleolar complexes purified by immunoaffinity selection with antibody to the protein Nop7p have been subjected to matrix-assisted laser desorption time-of-flight mass spectroscopy (MALDI-TOF MS) to reveal the amino acid sequences of the associated proteins, with the detection of both predicted and novel proteins (P. Harnpicharnchai from J. Woolford's group, Carnegie-Mellon University). Similar studies with mutants blocked in various steps in ribosome biogenesis were reported, linking proteomics to rRNA processing in a forward-looking way. In related studies, a yeast nucleolar substructure has been isolated and characterized with respect to nascent ribosomal particles (P. Milkereit from the laboratory of E. Hurt, University of Heidelberg). Two novel heterotypic protein complexes, one containing Noc1p and Noc2p and the other containing Noc2p and Noc3p, are involved in maturation of the 60S subunit. Another study exploited the differential association of the protein nucleolin with nucleoplasmic versus exported cytoplasmic ribosomes (F. Triolo from S. Pinol-Roma's laboratory, Mt. Sinai School of Medicine). These nucleolin antibody affinity selection experiments have revealed novel proteins that associate transiently with nascent ribosomes during their extranucleolar, pre-cytoplasmic phase of maturation in mammalian cells. One of the most well-known nucleolar proteins, B23, turns out to be a potent and (curiously) ATP-independent protein chaperone based on its antiaggregation activity against Rev, rhodanese, alcohol dehydrogenase, and citrate synthase (M. Olson, University of Mississippi Medical Center). The chaperone activity of B23 is a first for a nucleolar protein and the physiological relevance of this in vitro property is hinted by the finding that B23's phosphorylation by casein kinase II is enhanced when it is engaged by a denatured protein, leading to release of the bound substrate. One now awaits an implication of this intriguing property of B23 in an actual nucleolar function. Finally, a MALDI-TOF MS study of proteins from purified HeLa cell nucleoli has revealed over 300 proteins—some familiar, many others not, with detailed characterization underway (C. Lyon from A. Lamond's group, University of Dundee).

Several new findings on ribosome export from the nucleus were presented. In addition to involving the Ran-GTPase cycle, ribosome export in *S. cerevisiae* involves the Nsp1p/Nup82p/Nup159p complex (P.-E. Gleizes from the laboratory of I. Léger-Silvestre, CNRS and Université Paul Sabatier, Toulouse), Kap123p, and its novel binding partner Rbt1p (J.D. Aitchison, University of Alberta) and Nmd3p (G. Kallstrom, University of

Texas, Austin). In addition, mutants in the *S. cerevisiae* nuclear transport factors Srp1, Dap95, Pse1, Cse1, and Mtr10 cause 20S rRNA to accumulate in the nucleolus (T. Moy from P. Silver's lab, Harvard Medical School and Dana-Farber Cancer Institute). Finally, a new screen for ribosome export genes has revealed one that is an allele of RPL10, which not only encodes a late-assembling large subunit protein, but also, very interestingly, is involved in subunit joining (O. Gadal of E. Hurt's group, University of Heidelberg). Clearly, a large step in our overall understanding of ribosome export is, finally, about to be made as these genetic and biochemical studies reveal the factors involved in both nucleolar and nuclear export of ribosomes. Although not (yet) addressing specifically the export of ribosome cargo, a comprehensive, indeed elegant, analysis of the functional architecture of the nuclear pore complex (M. Rout, Rockefeller University) provided a beautiful context for the other ribosome export studies presented.

A number of presentations addressed the relatively new idea that the nucleolus may be doing more than just making ribosomes. The signal recognition particle RNA and three of its associated proteins have been localized in the nucleolus, and the nucleolar localization of SRP RNA is linked to both ongoing ribosomal RNA synthesis and the Ran-GTPase nuclear protein import/export pathway (T. Pederson, University of Massachusetts Medical School). Perhaps related, evidence for a secretory pathway-linked regulatory circuit feeding back on ribosome synthesis was presented (J. Warner, Albert Einstein College of Medicine). In a workshop invited by the meeting organizers, the idea that the nucleolus is plurifunctional was further advanced. These presentations included the findings that several tRNAs are processed in the nucleolus (D. Engelke, University of Michigan), that HIV mRNA localizes in the nucleolus (A. Michienzi from J. Rossi's group, Beckman Institute, City of Hope), that telomerase RNA is associated with the nucleolus (R. Terns, University of Georgia), and that spliceosomal U6 RNA transits through the nucleolus (S. Gerbi, Brown University).

Though not as strongly represented in the meeting as might have been ideal, evolutionary themes were occasionally on display. Two presentations on autogenous regulation of the S10 ribosomal protein operon by the large subunit protein L4 (J. Zengel and T. Allen, both with L. Lindahl, University of Maryland, Baltimore County) revealed the presence of this control mechanism in all enterobacterial species in the genome databases as well as the group containing *Vibrio cholerae* and *Hemophilus influenzae*, but not in the closely related group represented by *Pseudomonas aeruginosa*, nor in the more distantly related *Bacillus subtilis*. These results provide a fascinating window on the evolution of this control circuit, so well characterized in *Escherichia coli*, and points to its first occurrence in the gamma branch of proteobacteria. The presence of small nucle-

olar RNA homologs in the Archaea was demonstrated by immunoprecipitation of RNPs from *Sulfolobus acidocaldarius* with antibodies against the fibrillarin and Nop56 proteins of this organism (P. Dennis, University of British Columbia). The revealed snoRNA-like RNAs (hereafter termed snoRNAs for simplicity even though the Archaea, of course, do not have nucleoli, or even nuclei), had the characteristic C/D box motif and were 50–60 nt in length. Genes for these apparent snoRNAs were detected in seven other archaeobacterial species, indicating that they likely typify this major phyletic kingdom. These archaeobacterial snoRNAs have three very intriguing features: (1) some display regions of complementarity to tRNA, as well as to rRNA; (2) the number of snoRNAs in a given archaeobacterial species is curiously correlated with the optimum growth temperature; and (3) the putative guide regions of these archaeobacterial snoRNAs have diverged to a much greater extent than their corresponding rRNA targets. All three of these findings have far-reaching implications. Archaeobacterial snoRNA-ology now seems more exciting than even in the Eukarya.

Also in the spirit of evolution, the audience was reminded that mitochondria, of course, also make ribosomes, using proteins encoded by the nuclear genome and rRNA made within the organelle. The launch of a comprehensive (nuclear) genomics investigation of mitochondrial ribosomal protein genes was presented, with early indications of mutations relevant to human mitochondrial pathology (T. O'Brien, University of Florida, to whom we owe so much of what we know about mitochondrial ribosomes).

Once the rDNA and ribosomal protein genes have fired and the nascent subunits have assembled and conjoined, what then do we know of this machine, the ribosome? We now know very much indeed. The presentation, only days before its publication, of the 3.3 Å structure of the *Thermus thermophilus* small ribosomal subunit at the meeting was exhilarating (F. Franceschi, Max Planck, Berlin). The solved structure accounts for 1,457 of the 1,518 nt of the 16S rRNA and positions most of the contours of all 19 proteins of the particle. The structure was determined by multiple isomorphous replacement and anomalous scattering and is, like the extraordinary 2.4 Å structure of the large ribosomal subunit published the week before the Lake Tahoe meeting, a towering triumph of structural biology. The 3.3 Å small subunit structure presented at Lake Tahoe affirmed the protein-deficient motif of the large subunit region that faces the small subunit and reinforced the case for a RNA-based decoding center. Another feature of the 3.3 Å small subunit structure is an apparent latch between the protein-rich head and body of the particle, suggesting a clamp that grasps the mRNA. A major part of the recent excitement, of course, is the very fortunate fact that structure determinations of the two subunits have proceeded concurrently with that of

the 70S ribosome itself, for which Harry Noller and colleagues deserve the major credit. M. Yusupov (University of California, Santa Cruz) presented details of the 5.3 Å structure of functional 70S ribosomes, revealing the (mostly rRNA-interactive) tRNA A- and P-sites, the E-site, and the path of the mRNA. Fourier difference maps clearly establish the position of the Shine–Dalgarno interaction, the initiation site of mRNA base pairs with the 3' tail of 16S rRNA. The A- and P-tRNAs appear to interact exclusively with rRNA in the small subunit, but the E-tRNA also interacts with ribosomal proteins.

Though there was much at the meeting that was stimulating and provocative, I suspect that few in attendance would disagree that the presentations by Franceschi and Yusupov were two moments of absolute rhapsody. As I looked over to Professor Nomura

during these two talks I saw a broad smile on his face, summing up half a century, at this extraordinary moment in the history of molecular biology.

Hiking above Lake Tahoe (geologically cast ~70 million years ago) one marvels at its beauty. Then, upon reflection (no crystallographic pun intended), one remembers that ribosomes are, of course, much older (~3.5 billion years). They are thus all the more lovely to behold. And behold them we now can, at long last.

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