

What is life's secret? RNA! The ribosome teaches in silence.

Harry F. Noller

University of California, Santa Cruz, Sinsheimer Labs, Santa Cruz, CA 95064, U.S.A.

Ribosomes are responsible for protein synthesis, bridging the gap between genotype and phenotype. They are enormously complex macromolecular structures, containing the very large 16S and 23S ribosomal RNAs (rRNAs), 5S rRNA and more than 50 different proteins, with a total molecular mass of 2.5 MDaltons. The mechanism of action of the ribosome is beginning to be understood in terms of its molecular structure, which has recently been solved by X-ray crystallography. The three fundamental mechanisms of translation are aminoacyl-tRNA selection, catalysis of peptide bond formation (peptidyl transferase), and coupled translocation of tRNAs and mRNA following each round of peptide bond formation. Although the ribosome was once thought to be a passive surface upon which these functions are carried out by protein factors, it has become clear that these basic mechanisms are in fact embodied in the ribosome.

Biochemical, genetic and phylogenetic studies have provided evidence for the direct involvement of rRNA in these fundamental mechanisms. Moreover, it is likely that they are based on rRNA, rather than ribosomal proteins, consistent with the possibility that the first ribosomes were made exclusively of RNA. This can now be seen directly in the crystal structures, which show that the ribosome is basically an RNA complex, in which the majority of its proteins are attached to its exterior surface. The subunit interface cavity - the "active site" of the ribosome - is lined almost completely with rRNA.

The 5.5 Å structure of a functional complex of the complete 70S ribosome shows how the tRNAs are positioned within the ribosome, and which features of the ribosome interact with them. A Fourier difference map shows the path of the mRNA in the ribosome, including the Shine-Dalgarno interaction that is made between mRNA and 16S rRNA during translational initiation. The interactions made between 16S rRNA and 23S rRNA at the subunit interface are closely juxtaposed with the sites of contact between the ribosome and tRNAs, suggesting that movement of the tRNAs is coupled with rearrangement of inter-subunit contacts. Studies from the Ramakrishnan laboratory in Cambridge have shown how three conserved bases of 16S rRNA are able to monitor the accuracy of codon-anticodon interaction during tRNA selection. Moore, Steitz and their co-workers at Yale have shown that the peptidyl transferase center of the 50S subunit is made exclusively of 23S rRNA.

We are now entering a stage in which the dialogue between the structural work, including cryo-EM reconstructions as well as X-ray studies, and biochemical and genetic approaches, will bring us closer to understanding the molecular nature of the ribosome. Most important is the emerging realization that the ribosome is a dynamic object, a molecular machine based on RNA.

Continuous cell-free translation systems: history of the invention, reaction modes, and applications

Alexander S. Spirin

Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia

The idea that *in vitro* protein synthesis should be performed under conditions of continuous inflow of feed and removal of waste, rather than in a test-tube with a fixed capacity, was first realized in cell-free translation systems with matrix-immobilized template polynucleotides in flow-through columns (solid-phase translation systems) [1, 2]. Later it was discovered that a cell-free translation mixture could be confined within a flow-through cell by an ultrafiltration membrane, without immobilization on a matrix; in this case the flow fed the system and removed products, but the components of protein-synthesizing machinery were retained in the liquid-phase reactor [3, 4]. At about the same time (1988-1989) a simplified version of the continuous cell-free system was devised: incubation of the translation mixture in a simple dialysis bag was proposed for replenishing substrates and removing low-molecular-weight products by diffusional exchange across the membrane during the protein synthesis reaction [5]. Thus, the *continuous-flow cell-free* (CFCF) technique and the *continuous-exchange cell-free* (CECF) method were brought into use as two main formats of long-lived, high-productive continuous systems for *in vitro* protein syntheses. The methodology was applied to the synthesis of proteins both with prokaryotic and eukaryotic reaction mixtures, such as *E. coli* extract (ECE), wheat germ extract (WGE) and rabbit reticulocyte lysate (RRL) [3-6].

An important step in developing the continuous cell-free systems was the introduction of an mRNA-generating transcription sub-system into the reaction mixture. First a *coupled transcription-translation* with endogenous *E. coli* RNA polymerase was done in ECE using CFCF format [7, 8]. The next principal step was the creation of more effective *combined transcription-translation* systems when an exogenous bacteriophage (such as T7 or SP6) RNA polymerase was added to a eukaryotic extract (WGE) or lysate (RRL) to produce mRNA on DNA template *in situ* (in the absence of endogenous RNA polymerase activities) [6, 9].

Two Japanese groups succeeded in great improving the continuous cell-free protein-synthesizing systems, mainly through principal modifications of cell extract preparation and composition. Yokoyama, Kigawa and associates [10] developed the *E. coli* extract and achieved the highest productivity of the ECE CECF combined transcription-translation system. Endo with colleagues [11] found that careful removal of endosperm from wheat embryos prior to preparing the extract resulted in high stability and productivity of the CECF system, probably due to elimination of translation inhibitors, such as tritin (ribosome-inactivating protein of wheat), thionins and RNAases. As a result, now

many functionally active proteins can be synthesized in amounts of milligrams per ml during a day using the CECF systems of either prokaryotic or eukaryotic type.

The CECF format instruments for continuous transcription-translation based on the *E. coli* extract has been recently launched into the market by the Roche Diagnostics GmbH (RTS 500, RTS 9000, RTS ProteoMaster). Up to 5 mg of an individual protein per ml during 24 hours can be synthesized in their RTS 500 reactor with the proper reagent kit, and the synthesis of up to 150 mg totally has been claimed for the RTS 9000 reactor. The CFCF systems promise even higher yields and open wide possibilities for automation and computerization of the *in vitro* protein synthesis.

Generally, the cell-free technology for protein synthesis has several advantages as compared with the biotechnologies based on living organisms; these are speed and directness of all procedures, absence of constraints from a living cell, easiness of operator's control, purity of a product, and wide possibilities of product modifications. Among the most obvious applications of the cell-free gene expression technology are the following: (1) synthesis of cytotoxic proteins and polypeptides; (2) expression of unidentified open reading frames, functionally unstable or poorly expressible genes, and genes encoding for unstable products; (3) functional mapping of genomes through direct expression of genomic libraries; (4) synthesis of proteins with unnatural, chemically modified or isotope-labeled amino acid residues, including those for NMR spectroscopy; (5) synthesis of polypeptides and proteins, including direct expression of genomic libraries, for structural analyses (e.g., by NMR spectroscopy and X-ray crystallography); (6) *in vitro* protein engineering; (7) screening of engineered and theoretically designed proteins. Some of the above-mentioned applications are already successfully realized in several laboratories (see, e.g., [10, 12-14]), including users of the Roche RTS instruments (see, e.g., papers by Monchois et al., Betton et al., Cho et al., and Schraeml et al. in [15]).

Bibliography

1. NV Belitsina, AS Girshovich and AS Spirin (1973) Translation of resin-bound polynucleotide. *Doklady Akad. Nauk SSSR* **210**: 214-227.
2. NV Belitsina and AS Spirin (1979) Translation of matrix-bound polyuridylic acid by *Escherichia coli* ribosomes (solid-phase translation system). *Methods Enzym.* **60**: 745-760.
3. AS Spirin, VI Baranov, LA Ryabova, SY Ovodov and YB Alakhov (1988) A continuous cell-free translation system capable of producing polypeptides in high yield. *Science* **242**: 1162-1164.
4. VI Baranov and AS Spirin (1993) Gene expression in cell-free systems on preparative scale. *Methods Enzym.* **217**: 123-142.

5. YB Alakhov, VI Baranov, SY Ovodov, LA Ryabova and AS Spirin (1995) Method of preparing polypeptides in cell-free translation system. Foreign Application Priority Data: Dec. 22, 1988 [SU]; Jul. 31, 1989 [SU]. *US Patent* No. 5,478,730.
6. AS Spirin (1991) Cell-free protein synthesis bioreactor. In *Frontiers in Bioprocessing II* (P Todd, SK Sikdar and M Beer, eds.), American Chemical Society, Washington DC, p.p. 31-43.
7. VI Baranov, IY Morozov, SA Ortlepp and AS Spirin (1989) Gene expression in a cell-free system on the preparative scale. *Gene* **84**: 463-466.
8. T Kigawa and S Yokoyama (1991) A continuous cell-free protein synthesis system for coupled transcription-translation. *J. Biochem. (Japan)* **110**: 166-168.
9. VI Baranov, LA Ryabova, OB Yarchuk and AS Spirin (2002) Method of preparing polypeptides in a cell-free translation system. Foreign Application Priority Data: Jul. 31, 1989 [SU]; May 29, 1990 [SU]. PCT Pub. Date: Feb. 21, 1991. *US Patent* No. 6,399,323 B1.
10. T Kigawa, T Yabuki, Y Yoshida, M Tsutsui, Y Ito, T Shibata and S Yokoyama (1999). Cell-free production and stable-isotope labelling of milligram quantities of proteins. *FEBS Lett.* **442**: 15-19.
11. K Madin, T Sawasaki, T Ogasawara and Y Endo (2000) A highly efficient and robust CF protein synthesis system prepared from wheat embryos: Plants apparently contain a suicide system directed at ribosomes. *Proc. Natl. Acad. Sci. USA* **97**: 559-564.
12. KA Martemyanov, VA Shirokov, OV Kurnasov, AT Gudkov and AS Spirin (2001) Cell-free production of biologically active polypeptides: Application to the synthesis of antibacterial peptide cecropin. *Protein Expression and Purification* **21**: 456-461.
13. T Sawasaki, T Ogasawara, R Morishita and Y Endo (2002). A cell-free protein synthesis system for high-throughput proteomics. *Proc. Natl. Acad. Sci. USA* **99**: 14652-14657.
14. D Kiga, K Sakamoto, K Kodama, T Kigawa, T Matsuda, T Yabuki, M Shirouzu, Y Harada, H Nakayama, K Takio, Y Hasegawa, Y Endo, I Hirao and S Yokoyama (2002) An engineered *Escherichia coli* tyrosyl-tRNA synthetase for site-specific incorporation of an unnatural amino acid into proteins in eukaryotic translation and its application in a wheat germ cell-free system. *Proc. Natl. Acad. Sci. USA* **99**: 9715-9723.
15. AS Spirin, ed. (2002) *Cell-Free Translation Systems*, Springer Verlag, Berlin-Heidelberg-New York, p.p. 197-202, 219-225, 227-233, 235-246.

Homology modeling for the proteins of all the species genes and the data base: FAMS and FAMSBASE

Hideaki Umeyama

School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Full Automatic protein-homology Modeling System(FAMS) automatically makes three-dimensional coordinates of proteins which are unknown for three-dimensional structures based upon x-ray diffraction analysis and NMR experiments etc. The whole genes for 99 biological species have been searched to find the family protein sequences for which the 3-dimensional structures have been determined. About 50% of genes proteins were found to have smaller sequence alignment E-value than 0.001, the number of which is well known as the limited value to search the family proteins. The experimental production of various proteins, on the other hand, in the cell free system became possible from many studies by Prof. Endo et al. of Ehime University. From the national project of Japan for the structural biology, the 3-dimensional structures of 3000 proteins will be determined within these five years. At the same time, 3-dimensional coordinates near around those experimentally analyzed structures almost will be calculated by using our homology modeling program, FAMS. In the world, totally, 10000 proteins will be experimentally researched in these five years. As the results, all the protein sequences will be modeled using the FAMS program. If the target molecules for new drugs are proteins, all the protein structures will be obtained from the bio-informatical methods as shown in this lecture. In the future, accordingly, the bio-informatical researches will become very important to understand the biological systems and to find new drugs.

1. Ogata K. and Umeyama H.. (2000) An automatic homology modeling method consisting of database searches and simulated annealing, *J. Mol. Graph. Model.*, **18** (3), 258-272, 305-306
2. Iwadate M. , Ebisawa K. and Umeyama H.. (2001) Homology modeling of CAFASP2 competition, *Chem-Bio Informatics J.* **1** (4), 136-148
3. Yamaguchi A, Iwadate M, Suzuki E, Yura K, Kawakita S, Umeyama H, Go M. (2003) Enlarged FAMSBASE: protein 3D structure models of genome sequences for 41 species, *Nucleic Acids Res.*, **31**(1), 463-468

How protein factors control the catalysis of peptide bond formation by ribosomal RNA

Mathias Sprinzl

Laboratorium für Biochemie, D-95445 Bayreuth, Germany

The determination of the tertiary structure of the bacterial ribosomes revealed that the peptidyl transferase center of the ribosome consists of RNA [1]. It is not expected that proteins are involved in the catalysis of the peptid bond formation. However, the consecutive steps of initiation, elongation and termination are controlled by a complicated mechanism under participation of several translation (initiation, elongation and termination) factors. The determination of structure of these factors and the study of their mechanism, as a part of the complex ribosomal machinery, was the topic of our research in the past decade. The structures of elongation factor Tu, elongation factor Ts from *Thermus thermophilus* and several of their substrate complexes were determined by X-ray crystallography. In order to obtain information about the peptidyl hydrolase activity in the ribosomal peptidyl transferase center, we recently solved the structure of the *Thermus thermophilus* release factor 2 [H. Dobbek et al. unpublished]. Together with the available three-dimensional structures of other translation factors (EF-G, IF-1, IF3) in free and ribosome-bound forms, a picture of dynamic “ribosomes in action” starts to emerge. The structural studies on elongation factors were complemented by biochemical investigations. In this respect, the isolation of RNA aptamers that specifically interacts with translation factors and mimic the sites of interaction with ribosomal RNA provided valuable information about structural dynamic of the ribosomal A-site and the role of different 23S RNA domains (peptidyl transferase, α -sarcin/ricin domain, thiostrepton domain) during elongation and termination steps [2;3].

References

- [1] Cate, J.H., Yusupov, M.M., Yusupova, G.Z., Earnest, T.N., & Noller, H.F. (1999) X-ray crystal structures of 70S ribosome functional complexes. *Science*, **285**, 2095-2104.
- [2] Sprinzl, M., Hofmann, H.-P., Brock, S., Nanninga, M., & Hornung, V. (1999) RNA-Aptamers for studying RNA protein interactions. In *RNA Biochemistry and Biotechnology* (Barciszewski, J. & Clark, B.F.C., eds), pp. 217-228.
- [3] Szkaradkiewicz, K., Nanninga, M., Nesper-Brock, M., Gerrits, M., Erdmann, V.A., & Sprinzl, M. (2002) RNA aptamers directed against release factor 1 from *Thermus thermophilus*. *FEBS Lett.*, **514**, 90-95.

Characteristic features of mammalian mitochondrial translation systems and functional equivalency of mitochondrial tRNA and translation factors to *E. coli* counterparts

Kimitsuna Watanabe, Takashi Ohtsuki and Tsutomu Suzuki

Department of Integrated Biosciences, Graduate School of Integrated Biosciences, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba 277-8562, Japan

Animal mitochondrial (mt) translation systems possess a characteristic feature that RNAs are extremely simplified. Whereas more than 50 species of tRNA are required in usual bacterial and eukaryotic cytoplasmic translation systems, only 22 tRNA species are sufficient for translation of 60 codons in animal mitochondria, which are the minimal set of tRNA for decoding of the genetic code in extant organisms. In addition to unmodified U and G, only 4 kinds of modified nucleosides at the wobble position of tRNA anticodon (in which we found 3 novel modified nucleosides, 5-formylcytidine, 5-taurinomethyluridine and 5-taurino-methyl-2-thiouridine) are sufficient for discrimination of 60 codons.

Nematode mt translation system possesses another unique feature that 20 out of total 22 tRNA species are lacking in the T arm and the remaining two serine tRNAs are lacking in the D arm. We found two species of EF-Tu in nematode cell extract, one (named EF-Tu1) corresponding to the former T arm-lacking tRNAs and another (named EF-Tu2) to the latter D arm-lacking serine tRNAs. EF-Tu1 has an extra domain consisting of 57 amino acid residues in the C-terminal region, which seems to compensate for the missing T arm in these tRNAs to form a ternary complex among EF-Tu, aminoacyl-tRNA and GTP. EF-Tu2 turned out to be serine-specific, which has never been found in other organisms.

The mt ribosomes (mitoribosomes) are also characteristic. As compared with *E. coli* ribosomes, mt rRNA is shortened to about a half, instead protein contents are increased; the ribosomal proteins with binding sites on rRNA shortened or lost in the mitoribosomes are elongated as compared with the *E. coli* counterparts.

Exchange experiments of tRNA and translation factors between *E. coli* and mt translation systems (the latter of which was recently constructed by us) revealed that *E. coli* tRNA is functional in mt system and mt factors are available in *E. coli* system, suggesting their functional equivalency.

All these observations strongly suggest that mt proteins involved in translation systems have evolved so as to accommodate structural simplification of mt RNAs during the course of mt evolution.

Protein synthesis system as a nanomachine

Kin-ichiro Miura

Proteios Research, Inc. 1111 Tebiro, Kamakura-shi, Kanagawa 248-8555, Japan

The system for protein synthesis in a cell is a fine nanomachine. On the mechanism of protein synthesis, studies on the step of chain elongation preceded. However, studies on the steps of initiation and termination have progressed recently. As our research participates to the initiation step, especially focusing on the relating structure of the messenger RNA, the recent results are presented here.

There are differences in the structure of messenger RNA between prokaryote and eukaryote. As the informations of the total base sequence of genomes can be obtained, the frequency of base appearance in the 100 bases region before and after the initiation codon and the termination codon were studied. As for bacteria, the bar graph of bias from the base appearance frequency calculated by base ratio shows prominent peak of G at -10 position before the initiation codon, and broad distribution of A around there. This shows the existence of the Shine-Dalgarno sequence for ribosome binding. After the initiation codon, the biased appearance of A was found until the 10th codon. Also, before and after the termination codon, the appearance of A was biased. In eukaryote, the pattern of the bias of appearance frequency is different from the case of prokaryote. In the case of yeast, until -360 bases before the initiation codon, the appearance frequency of A is high, especially high at -3 base position. After the initiation codon, the large base bias is found only in the second codon. Several bases before and after the termination codons the appearance frequency of A is high. The effect of the base sequence after the initiation codon on the translation efficiency was examined by introducing mutations after the second codon of the *E. coli* dihydrofolate reductase (DHFR) gene, considering codon and base biases, and using *in vitro* and *in vivo* translation systems. In both assay systems, the two most frequent second codons, AAA and AAU, enhanced the translation efficiency several times compared with the case of the most common codon CTG, whereas the effects of lower frequency codons were not significant. Mutation in the third codon did not effect the translation efficiency. Therefore, the second codon in the bacterial mRNA seems to control the translation rate.

Most of eukaryote mRNA have the cap structure at the 5' -termini. The cap structure is bound to the initiation factor 4E in the protein synthesizing system. To see the interaction between the cap structure and eIF4E, recombinant human eIF4E was expressed in *E. coli*, purified and crystallized with the chemically synthesized cap structure m^7G^5, ppp^5, A or m^7GTP . The crystals were analysed by X-ray diffraction at 2.0Å resolution. And it was shown that the second nucleotide in the cap structure, p^5, A , as well as 7methyl guanylate residue m^7G^5, p acts important roles in the interaction with eIF4E. (Collaboration of Toshimasa Ishida's group)

Recently it was shown by biochemical experiments that the initiation factor eIF4E interacts also with eIF4G. eIF4G interacts with ribosome through other factors, and with the poly A binding protein, which interacts the poly A tail at the 3' -terminal region of eukaryote mRNA. Thus, the eukaryote polysome should take a circular shape. Already in 1997 we showed the circular polysome by mild treatment of protozoa *Tetrahymena* by electron microscopy and by scanning electron microscopy. Circular polysomes were detected in many kind of animal cultured cells by electron microscopy. (Collaboration of Kazumori Yazaki et al.)

Advances in cell-free protein synthesis system

Yaeta Endo

Cell-Free Science and Technology Research Center (CSTC), Ehime University, 3 Bunkyo-cho, Matsuyama 790-8577, Japan

Current cell-free protein expression systems are capable of synthesizing proteins with high speed and accuracy; however, the yields are low due to systems instabilities over time. Furthermore, existing systems are not suitable for expression of eukaryotic proteins. This report describes the highly efficient but also robust cell-free system for protein expression based on wheat embryos. We have initially investigated the source of instability of existing systems in light of endogenous ribosome-inactivating proteins and found that with respect to the wheat germ systems conventional wheat germ extracts contained RNA N-glycosidase tritin and other inhibitors such as thionin, ribonucleases, deoxyribonucleases, and proteases that originate from the endosperm and inhibit translation. Extensive washing of wheat embryos eliminates endosperm contaminants thus resulting in extracts with a high degree of stability and activity. In order to maximize the translation yield and throughput of the system, we have addressed and resolved the following issues: (1) optimization of the ORF flanking regions; (2) development of a new PCR-based approach for high-throughput mRNA production and expression screening and design and construction of expression vectors (peU vectors) containing different tags for large-scale protein production/purification. One possible procedure for the preparation of proteins in large amounts is as follows: (1) selection of suitable genes from a protein catalogue made by the PCR-directed system; (2) cloning into pEU vectors and transcription of mRNA; (3) fine tuning of translation conditions such as ions concentration and incubation temperature; and finally (4) protein synthesis in the dialysis reaction system.

The cell-free system, reported here, bypasses most of living processes and lends itself to a robotic automation for the high-throughput expression of genetic information, thus opening up many possibilities in the post-genome era.

Cell-free protein synthesis for structural proteomics

Takanori Kigawa¹ and Shigeyuki Yokoyama^{1,2,3}

¹*Protein Research Group, RIKEN Genomics Sciences Center, ²Cellular Signaling Laboratory, RIKEN Harima Institute at SPring-8, and ³Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo,*

¹ *1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa 230-0045, Japan*

² *1-1-1 Kouto, Mikazuki, Sayo, Hyogo 679-5148, Japan*

³ *7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

The RIKEN Institute has started the Structural Genomics/Proteomics Initiative (RSGI) (<http://www.rsgi.riken.go.jp>) at the Genomic Sciences Center (GSC) in Yokohama and the Harima Institute at SPring-8. RSGI is now integrated into the Japanese national project, National Project on Protein Structural and Functional Analyses, which has started fiscal 2002 to study both structures and functions of proteins in selected biological systems. We have been determining the three-dimensional structures of proteins of sequence families and analyzing molecular and cellular functions of these proteins in order to establish the structure-function relationships. Key technologies are essential to conduct the research in a high-throughput way. In particular, cell-free protein synthesis is highly suitable for high-throughput preparation and screening of protein samples for structural proteomics.

The system may produce proteins directly from PCR-amplified linear DNA fragments, requiring no cloning procedures. Hundreds of proteins and protein domains can be expressed from cDNA clones within a day. By using the dialysis method developed by Prof. Spirin, production of proteins has reached the level of milligram quantities. The system is useful to assess the solubility, productivity, and structural stability of proteins and thus practical for selecting protein samples for larger scale production. ¹H-¹⁵N HSQC spectra are measured to screen the domain/proteins, suitable for structure determination. His tag removal, changes in domain boundary, and optimization of the solvent condition may improve NMR spectra. In addition, it is successful in preparing labeled protein samples, the uniformly [¹³C, ¹⁵N]-labeled proteins for NMR structure determination and selenomethionine substituted proteins for X-ray crystallography. The robotic system has been adapted to advance the automated protein production.

The system accelerates high-throughput structure determinations by both X-ray crystallography and NMR spectroscopy. The large NMR facility with twenty NMR instruments of GSC is used for structural determination. A new facility with another twenty sets is being constructed. High-throughput Factory directed by Dr. M. Miyano has been established and two beam lines dedicated to structural genomics have been constructed at SPring-8 in Harima.

Challenges in the Age of Structural Proteomics

John L. Markley

Center for Eukaryotic Structural Genomics, Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, Wisconsin 53706, U.S.A.

We now have sequences of well over 22 million genes, yet we have structures for only ~18,000 proteins coded for by these genes. Why has it been so much easier to sequence genes than to determine three-dimensional structures and functions of proteins? In sequencing, the basic information needed is simply the linear order of four possible nucleotides. Sequencing is a linear activity in which fragmentary sequence information can be assembled to yield the whole. Proteins are synthesized as linear polymers, but require folding and proper conditions to achieve their active, three-dimensional states. Protein structures must be determined under native conditions by either X-ray crystallography or nuclear magnetic resonance spectroscopy. Both methods are time consuming, require large amounts of protein, and are fraught with uncertain success. Three-dimensional structures of proteins are extremely valuable in themselves and are the starting point for numerous directions in research and practical applications. Protein structures that reveal new folds or are associated with novel functions serve to map out new areas of sequence-structure-function space. Three-dimensional structures can provide clues to functions of unknown proteins and can serve as the starting points for understanding mechanisms of action and for designing drugs. The major challenges of structural proteomics are to develop broad-based technology for determining structures of proteins faster, cheaper, and better. We need better methods for predicting what proteins are ordered and what parts form stable domains. We need high-throughput methods for determining which proteins require binding partners to fold and become functional. We need methods for producing functional proteins faster and at lower cost. We need more efficient methods for determining three-dimensional structures. Finally, we need better ways of managing and exchanging information related to this enterprise. This lecture will survey the present state of the structural genomics field and discuss promising new developments from structural proteomics centers around the world that address these challenges.

[Supported by grant P50 GM64598 from the National Institutes of Health.]

Enhanced activity and stability of immobilized enzymes in functionalized nanoporous silica

Eric J. Ackerman

Biological Sciences Dept., Pacific Northwest National Laboratory, P.O. Box 999 / MS P7-56, Richland, WA 99352, U.S.A.

We have utilized functionalized mesoporous silica (FMS) with pore sizes in tens of nanometers, to pursue two highly efficient approaches for immobilization of proteins. Glucose oxidase (GOD), glucose isomerase (GI), and organophosphorus hydrolase (OPH) were either spontaneously entrapped or covalently linked in carboxylethyl- or aminopropyl-FMS with rigid, uniform open pore geometry. In the former case, when the enzyme was incubated with FMS, the protein molecules were sequestered in, or excluded from, the porous material, consistent with electrostatic interaction with the charged functional groups. The working buffer pH, concentration and ionic strength had an effect on the efficiency of protein entrapment and the resulting specific activity of the entrapped enzyme. In the latter case, FMS were first reacted with a bifunctional cross linking agent, thereby first forming one functional end covalently linked to the internal wall of FMS with its other end available for linking to the target protein. This procedure reduced or eliminated the possibility of intermolecular or intramolecular cross-linking of the protein by the cross-linking agent. By spontaneously entrapping or covalently linking, the immobilized enzyme exhibited comparable K_m and V_{max} to the free enzyme in solution, presumably due to the large open pore structure of the mesoporous silica. By using FMS, immobilization was much more efficient in terms of both amounts and activity of enzyme compared to either unfunctionalized mesoporous silica, or unfunctionalized/functionalized normal porous silica with the same pore size. In contrast to the same enzymes free in solution, the enzymes immobilized in FMS showed both higher specific activity and much enhanced stability. Surprisingly, inactive can be reactivated in the organically functionalized nanopores over storage. The combination of high protein loading, high immobilization efficiency and enhanced stability is attributed to contributions from the large and uniform pore structure, and to favorable environments introduced by the functional groups. In principle, this approach should be applicable to most enzymes, proteins, and protein complexes. The combination of FMS with the proteins offers an excellent platform for biological reaction engineering.

The current status of Japanese Bio-Industry and impact of Cell-free systems on it

Mitsuru Miyata

Nikkei Business Publications, Inc., 2-7-6, Hirakawa-cho, Chiyoda-ku, Tokyo 102-8622, Japan