

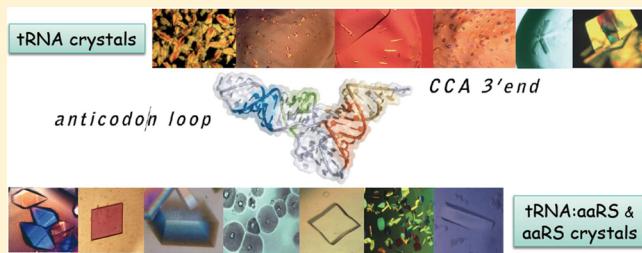
Crystallogenesis at the Heart of the Interplay between Science and Technology in the Quest to Comprehend tRNA Biology

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ABSTRACT: Transfer RNAs (tRNA) are small RNAs that provide the interface between DNA and ribosome-dependent protein synthesis, besides being involved in many other cellular processes. They interact with an impressive number of small molecule and macromolecular ligands and show structural and functional plasticity far to be deciphered. Here it is shown how tRNA biology was (and still is) idea and technology-driven and why crystallogenesis was at the heart of this science/technology interplay. Thus the quest to understand tRNA recognition/misrecognition by aminoacyl-tRNA synthetases (aaRS) stimulated biologists since the 1960s to crystallize tRNAs and their protein partners under their different functional states. This led to novel crystallization methods (vapor phase diffusion, microdialysis), characterization of system-specific additives (polyamines, metal ions, adenylate analogues), and discovery of ammonium sulfate as a crystallant for tRNA:aaRS complexes. Studies on the physical chemistry of tRNA and aaRS crystal growth, including the role of microgravity, were undertaken, and advanced methods for optimizing crystal quality were validated. As a result, the empiricism in tRNA crystallization was replaced by more rationality and led to the discipline of crystallogenesis. This facilitated tRNA crystallography, culminating with tRNA on the ribosome, thereby providing a robust structural background to better comprehend tRNA biology.



■ INTRODUCTION

Production of scientific knowledge often results from a mutual interplay between basic questioning and required technology. This is particularly true for the science of tRNA and the correlated development of dedicated methods for their structural and functional analyses. The tRNA molecules (Figure 1) are at the heart of biology with an origin deeply rooted in the tree of life. On the other hand, crystallization is the prerequisite for X-ray crystallography and often was a painful bottleneck that often found solution by chance or particular skills of the investigators. The urgent necessity of tRNA crystals in view of finding robust answers to many basic questions already raised at the time of nascent molecular biology explains the birth of biocrystallogenesis that developed in parallel with the progressive deeper understanding of tRNA biology. The 50 years of mutual enrichments of the two disciplines are outlined in this essay.

■ TRANSFER RNAs AT THE FOREFRONT OF MOLECULAR BIOLOGY

tRNA in Protein Synthesis and other Biological Processes. The participation of small RNA components as intermediates in protein synthesis (that later became known as the tRNAs) was suggested independently in 1954 by theoretical considerations of Crick in his “adaptor hypothesis” (published

as a letter to the members of the RNA Tie Club)² and by biochemical evidence from Zamecnik's laboratory indicating the presence of a fraction of small RNAs that did not centrifuge down with ribosomes in a cell free system in which amino acid activation occurred (first designated soluble RNAs or sRNA) (reviewed in ref 3). According to Crick's hypothesis, the RNA adaptors (in fact the tRNAs of much larger size, 75–90 nucleotides (nts), than the small RNAs originally proposed by Crick) would make the interface between mRNAs (carrying the genetic information from DNA) and proteins (in which this information is expressed). Explicit demonstration of the adaptor hypothesis⁴ was followed by many studies on tRNA function, purification, and sequence analysis (reviewed in ref 5) as well as by first crystallization attempts and determination of the crystal structure of tRNA^{Phe} from yeast (reviewed in ref 6).

Today it is well-known that all living organisms contain sets of 20 tRNA families, each specific for a given proteineous amino acid, accompanied by the cognate aminoacyl-tRNA synthetases (aaRSs). These enzymes attach the amino acids on the tRNAs. They are 20 in total in most organisms, each specific for one amino acid (notice that the non-discriminating AspRS and GluRS, present in a few Bacteria and in most

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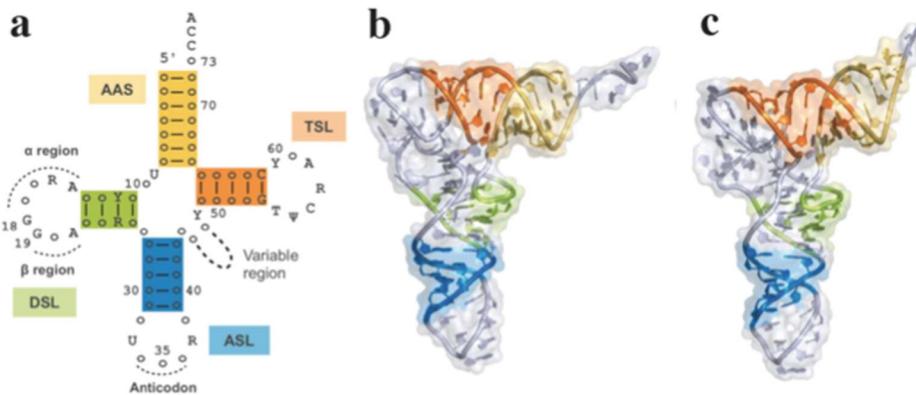


Figure 1. Seminal structural features in tRNA. (a) Generalized cloverleaf fold of tRNA (with standard numbering of residues). Conserved and semiconserved residues are indicated in bold (R for purine; Y, pyrimidine; T, ribothymidine; Y, pseudouridine); α- and β-regions (2–4 nts and 2–6 nts, respectively) in D-loops correspond to sequence stretches 5'- and 3'-apart from conserved G₁₈G₁₉; extra-sequences are inserted between residues 47 and 48 and form the extra arm in the variable region. Notice the presence of modified nucleosides in all tRNAs (almost 100 presently characterized). (b) L-shaped conformation of yeast tRNA^{Phe} (view computed with refined coordinates, PDB code 4tra). (c) Boomerang-like L-shape of yeast tRNA^{Asp} (closed form, PDB code 2tran). These conformations are those of native tRNAs with modified residues (11 in tRNA^{Phe} and 5 in tRNA^{Asp}). The four constitutive helical stems of tRNA are color-highlighted in the generalized 2D cloverleaf fold and 3D-foldings: yellow for the Amino acid Accepting Stem (AAS), orange for the T Stem Loop (TSL), green for the D Stem Loop (DSL) and blue for the Anticodon Stem Loop (ASL). See text and ref 1 for details.

Archaea, are specific for two amino acids, Asp/Asn and Glu/Gln, respectively.⁷ Then, an elongation factor carries the aminoacylated tRNAs to the ribosome where they interact via their triplet anticodon with the codons of mRNA (where genetic information of DNA is processed). The phenomenology of protein synthesis was already established in the early 1960s and since then became progressively sophisticated by integrating increasing structural knowledge (reviewed in refs 8–10). Unexpectedly, atypical functions of tRNAs and aaRSs, outside of protein synthesis, were discovered early on (e.g., a tRNA^{Gly} species participates in cell wall synthesis),¹¹ and their repertoire is continuously increasing (Table 1 and reviewed in refs 9, 12, 13). Evolutionary considerations and the ancient nature of tRNAs and aaRSs likely account for this diversity.

Fundamental Questions in tRNA Biology. Despite incredible advances, many questions about tRNA functioning (and malfunctioning) are only partly answered. In what follows, emphasis is given to those questions related with structural aspects. They are numerous, since during their cellular life

individual tRNAs interact with up to ~100 different macromolecular ligands, protein factors, nucleic acids, and/or enzymes (with ligands interacting either specifically with individual tRNAs or globally with groups of tRNAs). Questions related to the origin of life and genetic code expression during translation are the most fundamental. However, understanding the connection between the origin of tRNA and the origin of life remains elusive. Likewise the connection between genetic code and tRNA aminoacylation needs to be refined, in particular, the evolutionary and mechanistic impact of amino acid misincorporation into proteins that can be the consequence of false tRNA aminoacylations (frequently observed) as suggested already 50 years ago by an insightful *in vitro* experiment.¹⁴ The theoretical implications are the necessity of identity rules accounting for adequate levels of specificity of tRNA aminoacylation reactions^{15,16} and of editing mechanisms for error corrections.¹⁷ Related to these key problems about recognition by aaRSs is the quest to comprehend the mechanistic of the specific and nonspecific tRNA recognitions by its many other macromolecular ligands. Likewise correlations between structural and functional tRNA plasticity,¹ as well as between tRNA dysfunction and disease,¹⁸ have to be investigated more thoroughly. In another perspective, questions only marginally understood touching the biology of tRNA fragments (widespread in genomes and induced by cellular stress)^{19,20} and the molecular mimicry of RNAs or proteins with tRNA²¹ need more attention.

Table 1. Functional Diversity of tRNAs and Aminoacyl-tRNA Synthetases^a

tRNAs ^{8,9} (20 isoacceptor families)	aminoacyl-tRNA synthetases ¹² (18–22 species)
aminoacylation by aaRSs and donor of amino acids in protein synthesis	aminoacylation of tRNAs
biogenesis, maturation, degradation	editing of noncognate amino acids
reverse transcription	cytokine function
Asn and Gln biogenesis	aminoacyl-tRNA channeleing
tRNA-like structures in plant viral RNA genomes	viral assembly
cell wall synthesis	translational silencing
Leu/Phe addition on protein N-termini	splicing
porphyrin biosynthesis	antiapoptosis
recycling peptidyl-tRNA	other functions expected
other functions expected	

^a Seminal functions emphasized in bold.

CRYSTALS FOR IMAGING THE MACROMOLECULAR COMPONENTS OF LIVING ORGANISMS

The Art of Crystallizing Proteins. Before the advent of molecular biology in the 1950s, crystallization of biomolecules was an attribute of biochemists and physiologists (reviewed in ref 22). The story started in 1840 with the observation by Hünefeld of crystalline hemoglobin in desiccated human blood.²³ For about one century, crystallization became a means for protein purification and characterization. The situation changed when X-ray crystallography entered biology.

The first highlight arose in 1934, with the first X-ray photograph of a protein crystal²⁴ and was followed in the next decade by the establishment of methods for structure solving, in particular to overcome the phase problem.²⁵ Growing crystals was not a major concern for the first pioneers of structural biology who investigated proteins available in large amounts and rather easy to crystallize with bulk methods. The situation changed dramatically in the 1950s and 1960s when investigators became more ambitious. The supply of the interesting proteins became a limiting factor and the poor success rate in crystallization attempts led many scientists to consider protein crystallization as an art where magic skills of the investigators are essential for success (reviewed in ref 26). Fortunately this was not the opinion of a few pioneers who developed novel crystallization methods requiring less protein material.²⁷ Despite these advances, fabrication of crystals suitable for X-ray diffraction studies remained a major bottleneck in many objective-focused projects. The field of tRNA in the context of protein synthesis was probably the first emblematic objective-focused project that enlarged the problem of protein crystallization to large nucleic acids²⁸ and nucleoproteic complexes,²⁹ and thereby stimulated worldwide interdisciplinary efforts to overcome bottlenecks in the process of imaging the macromolecular components of living organisms (reviewed in ref 26).

Challenging Problems in Macromolecular Crystallization Seen from the Viewpoint of tRNA and Protein Synthesis. The first problem in the story of tRNA and aaRS crystallization was the choice of the adequate organisms from which the target molecules had to be purified. *Saccharomyces cerevisiae* and *Escherichia coli* were the first selected organisms, although as for aaRSs it was early on recognized that thermophilic organisms could provide more homogeneous protein samples, thus better suited for crystallization. The bacterium *Bacillus stearothermophilus* (renamed *Geobacillus stearothermophilus*) was first chosen³⁰ and progressively replaced by the more thermophilic *Thermus thermophilus* (reviewed in ref 31). In this context the history of ribosome crystallization is emblematic and clearly indicated that higher quality of ribosomes originating from organisms living under extreme conditions is associated with better crystals (reviewed in ref 32).

Free tRNAs. Preparation of pure tRNA species was a major concern (given that each organism contains ~40–60 individual tRNA members of likely similar structure) and required nonconventional purification methods such as countercurrent distribution and specialized column chromatographies (RPC, benzoylated DEAE-cellulose, Sepharose 4B eluted with reverse salt gradients). The fact that the crystal structure of yeast tRNA^{Phe} was first solved is explained by the ease to get large amounts of yeast cells and the straightforward preparation of the tRNA (only one step on BD-cellulose) in large amounts. This is due to the presence of the hydrophobic hypermodified Y-residue at position 37 of its sequence. Thus the gram amounts of more or less pure tRNA that were needed for the crystallization attempts could be isolated (with ~100 µg being a rough estimate of the amount pure tRNA needed for one assay). For other tRNAs, this facility does not exist and represented for a long time a bottleneck for structural studies (reviewed in refs 26, 33–35).

Because of such difficulties, some early crystallization attempts were undertaken with unfractionated tRNA preparations, assuming that for functional reasons all tRNAs should

have similar structures, an assumption that receive support by the growth of crystals from bulk yeast tRNA by an evaporation procedure.³⁶ Although biochemical data suggested the presence of multiple tRNA species in some crystals, it turned out that most of them contained predominantly tRNA^{Gly} (at least 60%).³⁷ Crystal analysis was however tediously difficult and the approach of bulk crystallization was abandoned. These precursory crystallization attempts, however, were not without interest, since they showed that purity of a tRNA sample is not an absolute necessity for crystallizability.

About 17 different tRNA species corresponding to 10 identities were crystallized in early times, all from *E. coli* (with Arg, fMet, Leu, Phe, Tyr, Val identity) and *S. cerevisiae* (Asp, Met, Leu, Phe, Ser, Thr, Val) (reviewed in ref 33). Crystalline polymorphism (~100 different crystal forms observed, mainly for *E. coli* tRNA^{fMet} and tRNA^{Phe} and yeast tRNA^{Phe} and tRNA^{Asp}) and poor diffraction properties were common features. As an example, Figure 2 shows two crystal forms of yeast tRNA^{Asp} (panels a and b). On the other hand, structural

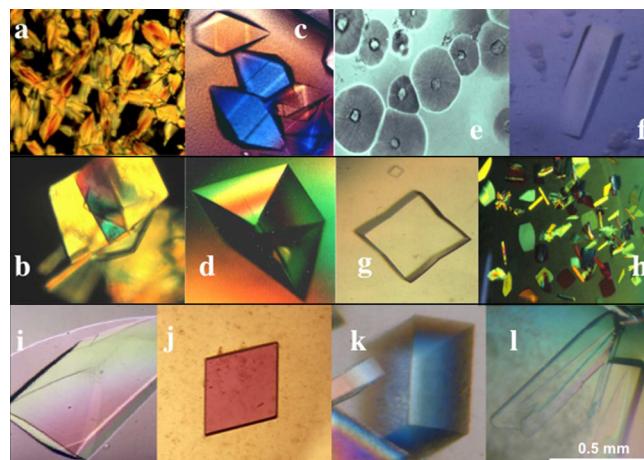


Figure 2. A gallery of tRNA and aaRS crystals from the aspartate system. (a, b) Free yeast tRNA^{Asp} in two orthorhombic C222₁ polymorphs diffracting (a) at 6.0 Å [isopropanol, pH 6.0] and (b) at 3.0 Å [ammonium sulfate, pH 6.0] resolution.³³ (c, d) Tetragonal P4₂1₂ bipyramidal [ammonium sulfate, pH 5.6] and trigonal prismatic P3₂1 crystals of truncated yeast AspRS-70 [ammonium sulfate, pH 7.8] diffracting at 2.5–2.7 Å resolution.⁴⁵ (e–h) Different crystal forms of the yeast tRNA^{Asp}:AspRS complex: (e) spherulites and (g) cubic I432 crystals diffracting at 7.0 Å [ammonium sulfate, pH range 7.8–8.5] (slight variation in the 2tRNA/aaRS ratio account for the growth of spherulites)⁴⁶ and (f, h) orthorhombic P2₁2₁ polymorphs diffracting up to 2.7 Å [ammonium sulfate, pH range 6.0–7.5] with (f) best crystals growing at pH 7.5.⁴⁷ (i–k) Crystals of *T. thermophilus* AspRS-1 (discriminating form specific for tRNA^{Asp}) diffracting at 2.65–2.2 Å and grown by an Ostwald-type ripening process,⁴⁸ with earth-grown (i) orthorhombic P2₁2₁ [ammonium sulfate, pH 6.9] and (j) monoclinic P2₁ [PEG8000, pH 7.8] polymorphs,⁴⁹ and space-grown (k) monoclinic polymorph twice as thick as controls prepared in parallel on earth.⁵⁰ (l) Crystals of *T. thermophilus* AspRS-2 (non-discriminating form aspartylating both tRNA^{Asp} and tRNA^{Asn}) prepared by macroseeding (orthorhombic P2₁2₁ crystals diffracting at 2.5 Å [PEG8000, pH 9.5]).⁵¹ All crystals were grown by the vapor diffusion method, except in space where dialysis was employed.⁵⁰ Note that best well-shaped crystal morphologies were observed for crystallizations conducted under reduced convection, either on Earth (j) in agarose gel⁴⁹ or in space (k) under microgravity.⁵⁰ Crystallants and pH of growth media are indicated in brackets.

Table 2. Comparative History of tRNA/aaRS Biology and Advances in Biocrystallogenesis Made by Scientists Working or Having Worked in the tRNA/aaRS Field

steps in biocrystallogenesis	year	a few highlights in tRNA and aaRS biochemistry/biology
first protein crystal (1840: hemoglobin in blood samples) ^{22,23}	1840–1954	
first diffraction image (1934: of pepsin) ²⁴		
bulk methods (1935–1954: batch, macrodialysis, evaporation...) ²²		
first single crystal of nucleic acid (1968: <i>E.coli</i> tRNA ^{fMet}) ²⁸	1955–1980	necessity of tRNA (1955–1958: prediction, characterization and adaptor role in protein synthesis) ^{2–4} first tRNA sequence (1965: yeast tRNA ^{Ala}) ⁵
tRNA crystallization by vapor equilibration (1968: sitting drop method) ⁶⁴		atypical functions of tRNA (1971: first example in cell wall biosynthesis) ¹¹
phase diagram (1972: for optimizing tRNA crystals) ⁶⁵ and microdialysis (1973: for aaRS crystallization) ³⁰		3D structure of a tRNA (1973–1975: yeast tRNA ^{Phe}) ⁶
incomplete factorial crystallization experiments (1979) ⁴¹		kinetic specificity of tRNA charging (1973: based on catalytic efficiency of cognate and noncognate aminoacylation) ⁶⁶
ammonium sulfate as crystallant of tRNA:aaRS complexes (1980) ²⁹ and first ribosome crystals diffracting at rather high resolution (1980) ⁶⁷		
birth of the ICCBM conferences (1984–1985) ⁶⁸	1981–1997	identity determinants in tRNA for aminoacylation (1988: <i>E. coli</i> tRNA ^{Ala}) ^{71,72}
crystallizability diagnostic by DLS (1986) ⁶⁹ and role of purity in protein and nucleic acid crystallization (1986) ⁷⁰		
sparse matrix method (1991) ⁷⁹ – microbatch crystallization under oil (1992) ⁸⁰		antideterminants (1988/1990: in tRNA ^{Ile} & tRNA ^{Asp}) ^{73,74} tRNA:aaRS 3D structures (1989–1992: <i>E. coli</i> tRNA ^{Gln} : GlnRS and yeast tRNA ^{Asp} :AspRS complexes) ^{75,76}
deliberate Ostwald ripening of a protein crystal (1996: <i>T. thermophilus</i> AspRS) ⁴⁸		classification of aaRSs in two groups (1990: based on sequence and catalytic site architecture of <i>E. coli</i> SerRS and yeast AspRS) ^{77,78}
first AFM picture of RNA crystals (1997: yeast tRNA ^{Phe}) ⁸³		human tRNAs in relation with pathologies (1993: short review about first examples) ⁸¹ editing in tRNA sequences (e.g., 1996: identity changes by editing) ⁸²
tRNA synthesis for high-throughput crystallization (2001) ³⁹	1998–2012	identity rules for tRNA aminoacylation (1998: general and idiosyncratic features) ¹⁶
strictly comparative analysis of space-grown and earth-grown crystals (2002: the case of <i>T. thermophilus</i> AspRS) ⁵⁰		aaRS paralogs (2005: review on atypical functions) ⁸⁴
microfluidic chips for crystallization and data collection (2009: based on counter-diffusion) ⁸⁷		human aaRSs in relation with pathologies (2007: the case of mitochondrial AspRS) ⁸⁵ & encoded aaRSs in viruses (2007: MetRS and TyrRS in giant mimivirus) ⁸⁶

flexibility and chemical fragility of tRNA represented other features that dramatically affect crystallization,³⁸ as well illustrated in the case of yeast tRNA^{Asp}. More recently, tRNAs could be produced rather straightforwardly by transcription from synthetic genes or by chemical synthesis, leading to the crystallization of tRNA species without their post-transcriptional modifications or to tRNA subdomains.^{1,39}

Aminoacyl-tRNA Synthetases. First aaRS crystals were grown in the decade 1970–1980 and concerned *E. coli* MetRS,⁴⁰ *B. stearothermophilus* TyrRS³⁰ and TrpRS⁴¹ as well as yeast LysRS,⁴² LeuRS,⁴³ and AspRS.⁴⁴ They were followed by the first crystals of a tRNA:aaRS complex, namely, that of aspartate in yeast.²⁹ Figure 2 shows some typical free or liganded aaRS crystals from the aspartate system (panels c–l). Most of them were obtained with ammonium sulfate as the crystallant in the pH range 5.6–8.5; however, the crystals that allowed researchers to solve the structure of *T. thermophilus* AspRS-2 were grown with PEG8000 at unprecedented pH 9.5. Noticeable, the first *E. coli* MetRS crystals were grown from a functional proteolytic fragment,⁴⁰ and presently crystallization of protein fragments is of common usage, especially in the field of aaRSs (see e.g. refs 45, 52, 53).

All early aaRS crystals were of rather poor quality for structure determination, and rapidly availability of protein samples became a limiting factor for optimizing their diffraction quality. Thus, protocols for aaRS preparation in large amounts were worked out^{54,55} but were challenged by the frequent proteolysis of the purified aaRSs. This was accompanied by batch-dependent heterogeneities as seen by PAGE and IEF in apparently pure samples, notably, AspRS samples and even in AspRS crystals.^{56,57}

Solution of some problems came in the 1980s when gene technology entered the theater of structural biology. But despite the greater ease to prepare wild-type or engineered versions of aaRSs from any organism provided their genes can be cloned, experimentalists were still faced with the difficulty to crystallize these proteins. This relies on the modular architecture of aaRSs, their structural plasticity and their tendency to aggregate when concentration increases. Many approaches have been tested to overcome these difficulties, the most promising consisting of stabilizing in the crystallization media an aaRS conformation by the addition of substrates or substrate analogues, such as stable adenylate analogues.³¹ A tempting recent approach expected to enhance aaRS

crystallizability by favoring specific lattice interactions was recently proposed. It is based on surface-entropy reduction at aaRS surfaces by introduction of a leucine half-zipper. The method has been successfully applied to a functional domain of *E. coli* AlaRS and yielded high-quality crystals.⁵⁸

tRNA in Complex with Macromolecular Partners. High salt concentrations were known to disrupt tRNA:protein complexes, and therefore were not used in the early attempts to crystallize tRNA:aaRS complexes.⁴⁶ Surprise was great when crystals of the tRNA^{Asp}:AspRS complex could be grown at high concentrations of ammonium sulfate²⁹ under conditions where the aaRS remains catalytically active.^{59,60} The choice of ammonium sulfate as a crystallant was suggested to us by an observation showing that tRNA affects the elution of aaRSs from Sepharose columns by reverse ammonium sulfate gradients.⁶¹ Rapidly, ammonium sulfate became the crystallant of choice for crystallizing tRNA:aaRS complexes,³¹ and its use was extended for crystallizing all kinds of RNA:protein complexes, notably, complexes of tRNA with elongation factor,⁶² and other macromolecular ligands such as processing/maturation enzymes, transformylase of methionyl-tRNA^{Met}, transamidosome factors, and ribosome.¹

Altogether many different tRNAs, either with or without modifications, could be crystallized in complex with proteins and in many cases complex formation facilitated crystallization by minimizing plasticity of the tRNA (and/or protein). Interestingly, advantage was taken of this feature to crystallize aminoacylated *E. coli* tRNA^{Cys} in complex with elongation factor and thus to solve the structure of this tRNA.⁶³

MUTUAL BENEFITS OF THE INTERPLAY BETWEEN CRYSTALLOGENESIS AND TRNA SCIENCE

Many advances in the science of crystallogenesis paralleled those in the science of tRNA and protein synthesis and were of mutual benefit for the two disciplines. This interplay is illustrated in Table 2 that sketches the history of macromolecular crystallization and tRNA/aaRS science.

Sample Requirements and Screening Crystallization Conditions. The fact that availability of tRNA or protein ligands was a limiting factor for crystallization encouraged experimentalists to develop new crystallization strategies requiring reduced samples of macromolecules. Miniaturization of crystallization arrangements was a first goal and resulted in the invention of the vapor diffusion method for growing *E. coli* and yeast tRNA crystals.⁶⁴ This was followed by the development of microdialysis and microbatch methods in the laboratory of David Blow when pioneering the crystallography of aaRSs.^{30,80} These methods are well suited for small volumes (from 50 μL down to 1 μL or less in microbatch). In its original version, vapor diffusion crystallization was conducted in sealed “sandwich” boxes in which 6–9 drops of 10–50 μL/drop (with tRNA or other macromolecules) sitting on a glass plate are equilibrated against a reservoir containing the crystallant at a higher concentration than that in the drops. Equilibration proceeds by diffusion of the volatile species until vapor pressure of the drops equals that of the reservoir. Microdialysis for aaRS crystallization was first conducted in the so-called “Cambridge buttons” (comprising 10–50 μL depressions filled with aaRS solutions and sealed with dialysis membranes). To reach supersaturation, buttons are immersed in sealed vessels containing the crystallant.

Micromethods allowed screening of many crystallization conditions, but given that crystallization is a multiparametric

process, it explains why it became rapidly apparent that an extensive survey of crystallization spaces is not feasible in practice because of limiting amounts of available macromolecules (note that gram amounts of pure tRNAs or aaRSs were required for the early structure determinations). Therefore, searching and screening specific additives to enhance crystallizability or to optimize crystal quality were important duties of crystal growers. Such efforts were guided by the knowledge of biochemical or biophysical properties of the entities to be crystallized. In the case of tRNA, polyamines and divalent cations were suspected and then found to be crucial for crystallization.^{64,65} Interestingly, scrutinizing by a phase diagram approach the effects of these additives on the crystallization of yeast tRNA^{Phe} showed that the values of the [spermine]/[Mg²⁺] molar ratio determines the space group in which the tRNA crystallizes.⁶⁵

Other strategies to discover crystallization conditions were based on statistical methods. A breakthrough came with the proposal of an incomplete factorial approach in view to find correlations between crystal quality and experimental crystallization parameters. TrpRS from *B. stearothermophilus*, a protein yielding many crystal polymorphs, was the test protein and gave promising results⁴¹ that were later exploited for the determination of a large set of structures of this aaRS under different functional states (reviewed in ref 53). A second significant breakthrough came in the 1990s from the laboratory of Sung-Hou Kim, a pioneer in tRNA structural biology, with the publication of the first screening kit to rapidly explore crystallization parameter-spaces.⁷⁹

Sample Quality Control. Since the beginning, the quality of samples was a major concern in macromolecular crystallization, especially in the tRNA and protein synthesis field. Therefore, access to analytical tools for assessing sample homogeneity was of utmost importance, in particular the tool of dynamic light scattering (DLS). Several scientists from the protein synthesis community adapted and miniaturized the method for that purpose^{69,88,89} and applied it to aaRSs and other factors from the protein synthesis machinery as a diagnostic tool of crystallizability.^{90–93} The case of human mitochondrial TyrRS is emblematic to illustrate the point. Indeed this aaRS has the propensity to aggregate what is harmful for crystallization. The problem was solved by a DLS-guided search of conditions leading to TyrRS monodispersity and hence to successful crystallization.⁹² Chemical purity as well is important⁷⁰ and is illustrated by a case study on the crystallizability of *T. thermophilus* AspRS-1. In that case minute amounts of protein impurities (less than 1% as seen by PAGE and identified by microsequencing and database mining) control the growth of the aaRS in either monoclinic or orthorhombic crystal forms, and only removal of the impurity by additional chromatographies allowed growth of the orthorhombic polymorph.⁹⁴

The Beneficial Role of Microgravity Projects and the Associated Technological Innovations. Many crystallization projects were sponsored by Space Agencies in the hope to relieve the bottleneck of crystallization in structural biology. Even though the expectation was only partly fulfilled (only a small number of the structures were solved from space-grown protein crystals), microgravity research had many indirect beneficial effects (notably by the support of basic ground research) and was essential for understanding the crystallization process of proteins. Moreover it led to significant technological innovations for the assessment of growth conditions and crystal

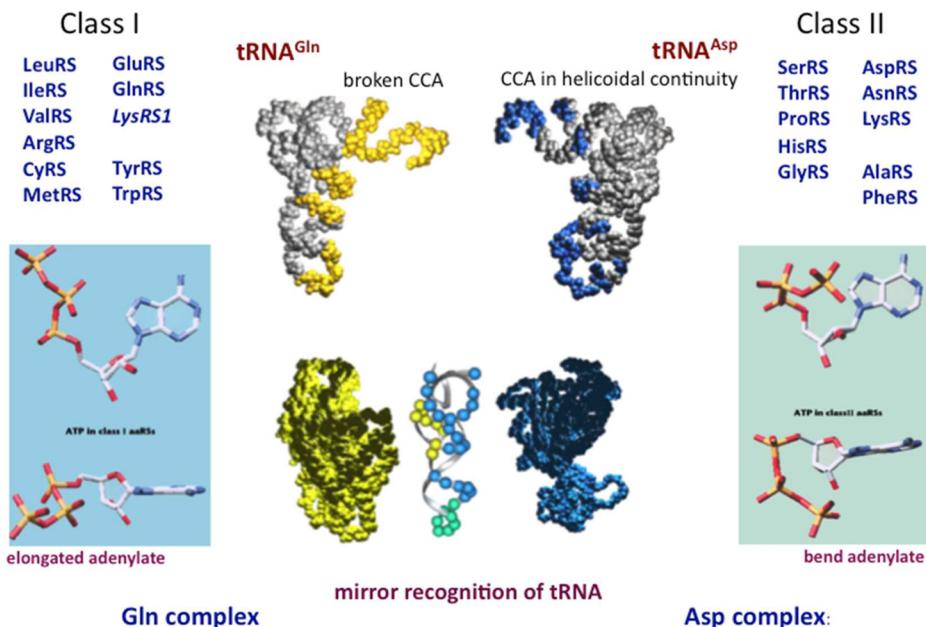


Figure 3. The universal conserved classes and subclasses of aaRSs with their main structural features emphasized, notably the ranking in two classes with three subclasses each, the different conformations of the ATP molecules when interacting with class I and class II aaRSs, and the aaRS class-dependent recognition-pattern of tRNA. (i) Class I aaRSs are characterized by a Rossmann-fold architecture in their catalytic domain replaced by an antiparallel β -sheeted architecture in class II aaRSs.⁸ Notice the presence of an atypical class I LysRS in a few Bacteria. (ii and iii) The most characteristic structural distinctions between the two classes of aaRSs are illustrated with examples from the *E. coli* glutamine (left) and yeast aspartate (right) systems. (ii) Class-dependent conformation of ATP and hence adenylate in the catalytic domains (displayed in two orientations rotated by 90°): in class I, ATP exhibits an extended conformation reminiscent of that found in other enzymes containing a Rossmann-fold, while in class II it adopts a bent conformation with the γ -phosphate folded back over the adenine base. (iii, top) Different orientations of tRNA bound to class I (broken CCA-strand) and class II (CCA-strand in helicoidal continuity with the AAS helix) aaRSs. In the glutamine complex,⁷⁵ 23 tRNA nts (in yellow) contact GlnRS (13 nts are identity determinants)¹⁰⁷ while in the aspartate complex,¹¹⁰ 19 tRNA nts (in blue) contact AspRS (6 nts are determinants).¹⁰⁸ (iii, bottom) Mirror recognition of tRNA as demonstrated by a “sandwiched” interaction potential between class I (ArgRS) and class II (AspRS) aaRSs and the same tRNA^{Asp} substrate with engineered arginine identity or aspartate identity.¹⁰⁹ Contacts with class I ArgRS (yellow spheres) and class II AspRS (blue spheres)¹¹⁰ are shown. Green spheres correspond to common contacts with either AspRS or ArgRS. Sketches of aaRSs are based on crystal structures of GlnRS and AspRS (only one subunit shown) and for clarity displayed translated away from the tRNA.

quality (reviewed in ref 95, 96). For instance it provided a driving force to improve the DLS technology, in scaling down volume of samples from mL to μ L ranges in the most advanced equipment.⁸⁹

As to biology-focused research, only a few projects were conducted with macromolecules from the protein synthesis machinery.^{97–99} We were contributors and among others made a rigorous comparison of *T. thermophilus* AspRS-1 crystals grown in parallel on Earth and in space. This demonstrated a reduction in mosaic spread in space-grown crystals,¹⁰⁰ resulting in a more accurate 3D-structure model at 2.0 Å resolution.⁵⁰ Furthermore it encouraged us to develop methods simulating in the laboratory conditions existing in space (i.e., reduced convection and sedimentation) either in gelled media¹⁰¹ or in microfluidic chips⁸⁷ based on the counterdiffusion approach.¹⁰²

■ CORRELATED ACHIEVEMENTS IN BOTH DISCIPLINES

Table 2 compares the advances in biocrystallography with those in tRNA science and shows how major biochemistry/biology results are correlated with achievements in the crystallography field. Because of space limitation only a few highlights are sketched in this essay (for more detailed information see refs 1, 8, 9, 53). The interplay between the two disciplines, with basic science and innovation in technology at the forefront, was possible thanks to the dedication of many

scientists (such as Christian Betzel, David Blow, Charly Carter, Brian Clark, Rolf Hilgenfeld, Sung-Hou Kim, Alex McPherson, Jo Ng, and Ada Yonath) who were formerly and/or still presently active in both biocrystallography and protein synthesis research.

The resolution of the two first crystal structures of tRNA^{103–105} was a first breakthrough directly correlated with progress in biocrystallography. An essential output was the discovery in these structures of networks of tertiary interactions including the conserved and semiconserved residues that form a compact framework accounting for their L-shaped architecture. Similar networks were found in other tRNAs and their 3D-organization could be rationalized recently.¹ The conclusions apply also to the atypical tRNAs found in mitochondria and account for the structural idiosyncrasies found in the tRNA world.¹ This conserved structural organization confers an intrinsic flexibility to tRNA molecules that relies principally on a rather weak connection between the D- and T-loops leading to potential bending motions around the elbow of the L. In a broader perspective, non-Watson–Crick base-pairings as found in tRNA were instrumental to understand RNA structures in general.¹⁰⁶

A second breakthrough was instigated by the resolution of the first crystal structures of tRNA:aaRS complexes.^{75,76} It arose as a multipartite interplay between crystallography, crystallography, enzymology and molecular biology (Table 2). Main

conclusions are illustrated in Figure 3. Thus and completely unexpected was the partition of aaRSs in two classes.⁷⁸ This was a direct offspring of the crystallographic investigations in the tRNA/aaRS field and was revealed by unprecedented features in the *E. coli* apo-SerRS⁷⁷ and the yeast tRNA^{Asp}:AspRS complex.⁷⁶ Importantly, this showed that the apparently degenerated signature motifs of class II aaRSs (e.g., only one strictly conserved proline residue in motif 1) could not be discovered by sole sequence analysis but necessitated 3D-knowledge. The second surprise was the different mode of tRNA interaction with class I and class II aaRSs. Although the two tRNA extremities make specific contacts with the aaRSs via their identity bases (except in tRNA^{Ala}), the way the interaction occurs with the catalytic domain of the aaRS is different. In the case of the class I glutamine complex it requires an opening of the last base-pair accompanied by a hairpin turn of the 3'-single-stranded acceptor CCA-end,⁷⁵ while in the class II aspartate complex the CCA-end remains in helicoidal continuity with the accepting helix.⁷⁶ The resulting mirror recognition of tRNA imposes two types of binding of the ATP/adenylate ligand in the catalytic site. Altogether tRNA interaction with aaRSs requires structural plasticity of both partners, a fact amply demonstrated by the presently available tRNA and aaRS crystallographic structures (reviewed in refs 1, 31, 53). This structural plasticity (as well as that of aaRSs) is a biological necessity imposed by the rich interaction potential of tRNAs with their many macromolecular partners⁹ and by the nonabsolute specificity of tRNA recognition by aaRSs implying possible aminoacylation errors.⁶⁶ This feature likely is a motor of evolution, since such errors can lead to false amino acid incorporations into proteins thereby creating diversity in proteomes.⁵³

The most recent breakthrough concerns ribosomology,³² and the fate of tRNA during peptide bond formation. Crystallography clearly shows that tRNA keeps its L-shaped conformation in its three binding sites (A, P and E) on the ribosome,^{111,112} although with faint conformational changes when compared with free tRNA.^{113,114} In this context deciphering the functioning of the Hirsh suppressor tRNA (an *E. coli* tRNA^{Trp} variant harboring a G24A mutation) was of particular interest, with the demonstration that reading of the UGA stop codon was due to increased flexibility of the mutant tRNA on the ribosome.¹¹⁵

PERSPECTIVES

Conformational plasticity and dynamics best characterize functional tRNA. This brings to the question of decrypting the kinematic changes in tRNA structures in action. The crystallographic snapshots obtained during the maturation of a tRNA was a first attempt toward this goal.¹¹⁶ Likewise, deciphering the structural role of modified residues in tRNA biology (e.g., in codon discrimination on the ribosome)¹¹⁷ will be important. The difficulty to prepare post-transcriptionally modified tRNA molecules for crystallization purposes explains why this topic was neglected. On the other hand interdisciplinary approaches, where biological and biophysical methods will guide the design of high-value particles to be crystallized, should be pursued in the tRNA field. The recent crystallographic work on human TrpRS crystals that allowed researchers to link cadherin biology with angiogenesis illustrates the point.¹¹⁸ Finally, the different structural signals in a given tRNA that specify its various functions (see Table 1) need to be deconvoluted, as well as those leading to tRNA dysfunction,

notably in human diseases, such as in pathogenic mutants of human mitochondrial tRNA^{Tyr} associated with ophthalmoplegia.¹¹⁹ That many of these signals are expressed at the 3D-level implies production of adequate crystals for structure determinations.

In conclusion I anticipate that crystallogenesis in the tRNA field will remain flourishing since tRNA not only is a model for understanding RNA structures and the rules underlying formation of protein/nucleic acids assemblies but also has a key position in biology.

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Notes

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