Elucidating the medium-resolution structure of ribosomal particles: an interplay between electron cryomicroscopy and x-ray crystallography

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Introduction

Ribosomes are the universal cellular organelles that accomplish the translation of the genetic code into proteins. Electron cryomicroscopy (cryoEM) has yielded fairly detailed three-dimensional reconstructions of ribosomes. These were used to assist in the determination of higher resolution structures by x-ray crystallography.

Results

Molecular replacement studies using cryoEM reconstructions provided feasible packing schemes for crystals of ribosomes and their two subunits from *Thermus thermophilus*, and for the large subunits from *Haloarcula marismortui*. For the large subunits, these studies also confirmed the major heavy-atom sites obtained by single isomorphous replacement combined with anomalous diffraction (SIRAS) and by multiple isomorphous replacement combined with anomalous diffraction (MIRAS) at approximately 10 Å. Although adequate starting phases could not be obtained for the small subunits (the crystals of which diffract to 3.0 Å), cryoEM reconstructions were indispensable for analyzing their 7.2 Å multiple isomorphous replacement (MIR) map. This work indicated that the conformation of the crystallized small subunits resembles that seen within the 70S ribosomes. Subsequently, crystals of particles trapped in their functionally active state were grown.

Discussion

Single-particle cryoEM can contribute to the progress of crystallography of nonsymmetrical, large, and flexible macromolecular assemblies. Besides confirming heavy-atom sites obtained from flat or overcrowded difference Patterson maps, the cryoEM reconstructions assisted in the elucidation of packing arrangements. They also provided tools for the identification of the conformation within the crystals and for the estimation of the level of inherent nonisomorphism.