Crystallographic study of *Thermus thermophilus* Initiation factor 2

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Abstract An essential step for accurate protein synthesis is the right placement of mRNA and tRNA in the initiation complex 30S. In prokaryotes system, this process requires three initiation factors: IF1, IF2, and IF3. The biggest of these three factors is IF2, a GTP/GDP binding protein of 63Kd (in *Thermus thermophilus*) that catalyzes the binding of initiator fMet-tRNA in the ribosomal P site in frame with mRNA. As a results of its activity the rate of translation initiation is increased and its fidelity is assured.
Despite the structure of all the other prokaryotic translation initiation factors have been resolved (IF1, Sette et al. 1997; IF3, Biou et al. 1995), as well as elongation factors EF-Tu and EF-G in various functional states (Kjeldgaard and Nyborg 1992; Berchtold et al. 1993; Kjeldgaard et al. 1993; Ævarsson et al. 1994; Czworkowski et al. 1994; Nissen et al. 1995, 1999; Abel et al. 1996; al-Karadaghi et al. 1996; Polekhina et al. 1996) and the ribosome recycling factor (RRF; Selmer et al. 1999), and translation termination factor RF2 (Vestergaard et al. 2001), until now no structure have been pubblished for prokaryotes IF2 alone or in its functional complexes. **Here we present preliminary results of initial crystallization trials of *Thermus thermophilus* IF2, showing that its crystallization is feasible and that at least in one cases a crystal was diffracting to 3.5 Å resolution**

Initiation of translation in bacteria

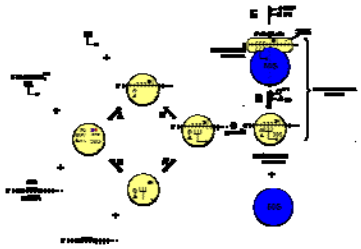


Fig 1. Schematic representation of the pathway of 70S initiation complex formation in eubacteria. See text for more details. Adapted from Gualerzi and Pon 1990.

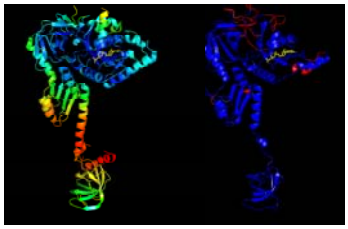


Fig 2. The three dimensional structure of the protein as predicted by homology modeling (Guex and Peitsch, 1997) with the archaeal IF2/eIF5B 3D structure (Roll-Mecak et al., 2000).
The regions of likely structural discrepancy between bacterial and archaeal proteins, according to the structural alignment and energy minimization, are shown in red. Bound GDP is shown in yellow.

CHALLENGE. Determination of three-dimensional structure of *Thermus thermophylus* IF2 require large good-quality crystal. Strategy consist in three important steps: purity protein, crystallization trials and identification of ideal precipitation agents.

More then 20 years of attempts did not produce any crystal structure for IF2.

1) N-domain is highly flexible.

2) Also the connection between C-1 and C-2 domain could be extremely flexible.

Protocol purification *Thermus thermophylus* IF2

Q-sepharose Gradient 0-100%
Amm.sulfate

Heating 65°C 20' and chilling ice 20'
Phenil-sepharose Gradient 85-0%
Amm.sulfate
BTP650S Gradient 10-100%
amm. sulfate

Gel filtration

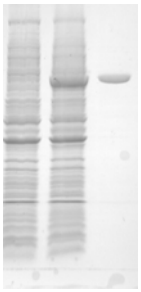


Fig 3. SDS-PAGE analysis of the purification of *Thermus thermophilus* IF2 expressed in *E. Coli.* The figure shows a scanned image of the Coomassie blue-stained gel. Lanes: 1, cell extracted of *E. Coli* BL21 before IPTG induction; 2, two hours IPTG induction; 3, purified IF2 from *Thermus thermophilus.*

Type of screening:

Cations	Sparse matrix
AMSO	MPDs
Natrix/Memfac	Index
Peg/ion/ph	Peg/ion
SaltRX	Wizard I and II
HS1/HS2	

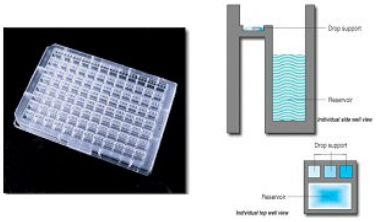
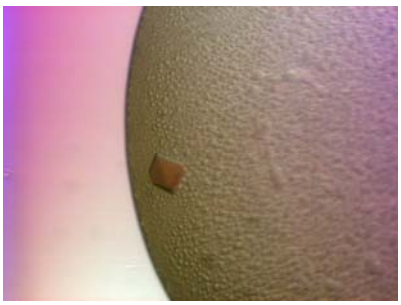


Fig 4. Crystallization set-up is done using a Tecan Workstation 150 robot mainly using the 96-well plate with square drop support from Greiner Bio-one in vapor diffusion technique

Result. Identification good crystallization conditions

Needles different shapes using different precipitant agents
One crystal square in Peg/ion A1 condition
Crystal was diffracting to 3.5 Å resolution
Crystals had grown also in presence of Ammonium sulfate and sodium citrate



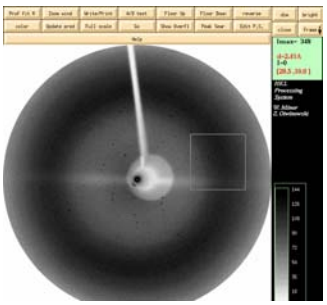


Fig 5. Crystal of *Thermus thermophylus* IF2 grown in the Peg/ion screening by vapour diffusion technique and its X-ray diffractogram

Cell parameters of IF2 crystal:

Cell (Å), a 45.80 b 61.89 c 162.85
alpha 90.00 beta 90.00
gamma 90.00

Space group (Å) P222

Diffraction limit 3.5 (Å)

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