Structural studies of the eukaryotic ribosomal stalk components

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The ribosome has a distinct lateral protuberance, the so-called “stalk”. This structure consists of highly conserved small (11 kDa) ribosomal proteins with an isoelectric point in the very acidic range (1). In eucaryotic cells, they are categorized into two groups, P1 and P2. The “stalk” is located in the active part of the ribosome structure where interactions between mRNAs, tRNAs, and translation factors take place during protein synthesis. The group of P-proteins is made up of a different number of components depending on the species. Thus higher eukaryotic cells, such as mammals, have two acidic ribosomal P-proteins (P1 and P2), plants have four (P1, P2a, P2b and P3), and lower eukaryotes such as Saccharomyces cerevisiae also have four (P1A, P1B, P2A and P2B) (2). Three distinct regions can be distinguished in the primary structure of P-proteins. Firstly, the amino-terminal region, containing a hypothetical bilateral hydrophobic zipper, is responsible for the dimerization of P-proteins and for anchoring them into a ribosomal particle. Secondly, the central part consists of a very flexible structure made up exclusively of alanine, glycine and proline. Thirdly, an intriguing C-terminal eleven amino acid sequence is extremely well preserved in all eukaryotic organisms, from yeast through humans, and only small alterations in this sequence are found. The high degree of conservation of this region suggests that it must play an important physiological role, but its exact function has not been evaluated as yet (3). The biochemical studies dealing with the three-dimensional structure indicate that single P-proteins in vitro may have a flexible structure, with some of the characteristics of a so-called molten globule (4,5). Studies on protein-protein interactions between P-proteins revealed that these particles preferentially form a P1-P2 hetero-complex (6). It is thought that the P-proteins are able to gain a stable 3D structure upon binding to each other, and presumably the hetero-dimer is an intrinsic form of these polypeptides (7). The 3D structure of the P-proteins has not been determined so far; nevertheless, the tertiary structure of the stalk was recently observed using cryoelectron microscopy at 17.5 Å resolution. The stalk has an elongated structure only when forming a complex with elongation factor 2 and with sordarin which seems to immobilize this complex. The prokaryotic ribosomal stalk structure, equivalent to the eukaryotic one, is formed by protein L7/L12. The structure of L7/L12 protein has been studied in great details, showing that C- and N-terminal domains are well defined compact structures joined by a “hinge” region (1). Several studies have shown that the “hinge” is unstructured and its flexibility is essential for protein activity (8-10); moreover, this is the only region to show some similarity to the “hinge” of eukaryotic P-proteins; except that there is no significant similarity in the primary structure between two polypeptides from both kingdoms. The main objective of the project is to determine the tertiary structure of the eukaryotic ribosomal “stalk” components, using SAXS (Small-angle X-ray scattering). The prokaryotic structure has attracted a lot of attention for many years and has been very well characterized. The eukaryotic “stalk”, in spite of its functional similarities to the prokaryotic one, is totally different in terms of primary structure and possesses a more differentiated function. Its exact functional role has not been clarified to date and solving its tertiary structure will cast more light on the modus operandi of this particular ribosomal component.

For analysis we have used yeast S. cerevisiae as well as human complexes. In the case of yeast, we analyzed P1A-P2B hetero-complex YHR, and its truncated form, where flexible C-terminal parts were removed from both proteins. The distance distribution function p(r) of YHR suggests that the particle has very elongated shape with the maximum diameter of about 10 nm and a cross-section of about 2.5 nm. The truncation mutant has the same cross-section but is significantly more compact having the maximum size of about 7 nm. Low resolution models of the full-length protein and of the deletion mutant were reconstructed ab initio from the experimental scattering patterns. The
reconstructions using the program GASBOR yielded superimposable results always providing good fits to the experimental data. The \textit{ab initio} models of the full-length YHR reveal two separated domains, (presumably, C-termini), possibly due to the flexibility of the C-terminal parts. As seen from the overlap, the shape of the truncation mutant can be well fitted into the central part of the YHR heterodimer model (Fig. 1)

![Ab initio models of the YHR heterodimers](image)

\textbf{Figure 1: Ab initio models of the YHR heterodimers.} Left pair: averaged shapes of the full length protein (left) and of the truncation mutant (right), right pair: corresponding most typical dummy residues models. The bottom row is rotated clockwise by 90° around the X axis.

In the case of human hetero complex, we used P1-P2 hetero-dimer and P2-P2 homo-dimer. In both cases the distance distribution function indicates an elongated particle with the maximum diameter about 11 nm, and a cross-section of about 2.5 nm. A shoulder at higher intraparticle distances observed in the p(r) function suggests that the average separation between the centers of monomers is about 6 nm. Low-resolution shape reconstructions of P1-P2 and P2-P2, performed both assuming P2 symmetry and without symmetry restrictions, lead to superimposable shapes. The averaged and the most typical \textit{ab initio} models for both dimers are very similar to the yeast structure, showing two linearly positioned monomers with a waist in the middle.

\textbf{References}