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Two-photon fluorescence microscopy *in vivo* studies of GFP-yeast ribosomal stalk proteins

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The S. cerevisiae ribosomal stalk, an essential large subunit protuberance, is made of a 34 KD protein, P0, and four 12 KDa acidic proteins, P1α, P1β, P2α and P2β *In vitro* results indicate that in purified ribosomes the four acidic proteins are present as monomers, which can form two preferential associations, P1α-P2β, P1α-P2β. Nevertheless, it has been shown that free P2 proteins can form dimers in solution, and unpublished cross-linking data suggest the existence of P2 dimers in the particles. To test the composition of the stalk inside cells, P0 and the four acidic proteins were GFP tagged, and expressed in yeast strains lacking the corresponding wild type proteins. The four single disrupted strains and all possible double disruptants were prepared. The transformed strains were studied by two-photon fluorescence microscopy and data analyzed using photon-counting-histogram (PCH) method, which allowed estimation of GFP molecules per particle. Using P0-GFP as a reference, the number of tagged acidic proteins associated to ribosomes was estimated. The results show that in cells expressing one tagged acidic protein only one GFP molecule is associated to the ribosomes. When two tagged acidic proteins were introduced simultaneously two GFP molecules were detected, except in the case of the cells expressing P2β and P1α at the same time. The results strongly support that the eukaryotic stalk in wild-type ribosomes is indeed made of protein monomers inside the cell, though when more than one acidic protein are missing two copies of the same protein can bind to the particles.