Alberto Garcia-Marcos, Susana A Sánchez, Pilar Parada, John S Eid, Enrico Gratton, Juan P G Ballesta, and David M Jameson.

Fluorescence Correlation Spectroscopy in vivo Studies of GFP-Yeast ribosomal stalk proteins.

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Abstract

GFP was linked to the C-terminal domains of Saccharomyces cerevisiae ribosomal stalk proteins, P0 and the acidic proteins P1a, P1b, P2a and P2b. Mutant yeast strains lacking one or all the possible combinations of two acidic proteins were constructed and the recombinant GFP fusion proteins were transformed into strains lacking the corresponding native proteins. The fluorescent proteins were incorporated into ribosomes and were studied both in vivo and in vitro using two-photon fluorescence correlation spectroscopy (FCS). The FCS data were analyzed using the photon-counting-histogram method which allowed for determination of the intrinsic “brightness” of the labeled ribosomes, which is related to the number of GFPs per particle. Using the P0-GFP construct as a reference, we were able to determine the number of P1a-GFP and P2b-GFP proteins associated with ribosomes in vivo. The results showed that only one GFP was found to be associated with each ribosome when only one labeled protein is present, indicating the labeled proteins are totally functional in the ribosome assembly process. In other cases two labeled acidic proteins were simultaneously expressed in the cells. In these cases two GFP constructs were found to be associated with each ribosome except in the case in which P2a and P1a were expressed simultaneously, probably because the GFP moiety may interfere with the incorporation of these two proteins, and suggesting that they are neighbors in the stalk. The overall results suggest that the yeast stalk composition is P0-P1a-P1b-P2a-P2b, excluding the possibility of the presence of homodimers in the ribosome. This ... [truncated at 250 words]