DOI: 10.46991/JISEES.2025.SI1.016

The Yin-Yang of the Green Fluorescent Protein: Effect on Stress Resistance in *Saccharomyces cerevisiae*

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ABSTRACT

Fluorescent biomarkers are widely used in cell biology to study gene expression and protein localization. Translational fusions, where a fluorescent protein is directly linked to a protein of interest, allow researchers to monitor subcellular distribution, while transcriptional fusions are used to assess promoter activity. Despite their extensive application, the potential physiological impact of these fluorescent tags on host cells remains largely overlooked. In this study, we investigated how the Green Fluorescent Protein (GFP) influences stress responses in the yeast *Saccharomyces cerevisiae*. We generated translational fusions of GFP with two proteins: Pab1p, a key component of stress granules, and Sur7p, a membrane-associated protein involved in the organization of Can1-enriched plasma membrane domains. These targets were selected because the cellular behavior of *S. cerevisiae* under varying heat and oxidative stress conditions remains incompletely understood. Our main findings indicate that the Pab1p-GFP fusion confers increased resistance to heat shock compared to the wild-type strain. Furthermore, strains expressing GFP-tagged proteins displayed altered cultivability under oxidative stress, suggesting that the presence of GFP can modulate the cellular stress response. *In silico* structural analysis confirmed that GFP fusion does not alter the overall 3D structure or function of the tagged proteins. This suggests that the observed phenotypic differences are likely due to the intrinsic properties of GFP, particularly its known ability to scavenge reactive oxygen species (ROS). These results highlight an important consideration for researchers using fluorescent tags: while GFP is generally considered a neutral reporter, it can influence cellular behaviour under stress, potentially affecting the interpretation of experimental outcomes.

Keywords: yeast, fluorescent protein, heat stress, oxidative stress, ribonucleoprotein, membrane patches

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