Analysis of deletion phenotypes and GFP fusions of 21 novel Saccharomyces cerevisiae open reading frames

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As part of EUROFAN (European Functional Analysis Network), we investigated 21 novel yeast open reading frames (ORFs) by growth and sporulation tests of deletion mutants. Two genes (YNL026w and YNL075w) are essential for mitotic growth and three deletion strains (ynl080c, ynl081c and ynl225c) grew with reduced rates. Two genes (YNL223w and YNL225c) were identified to be required for sporulation. In addition we also performed green fluorescent protein (GFP) tagging for localization studies. GFP labelling indicated the spindle pole body (Ynl225c-GFP) and the nucleus (Ynl075w-GFP) as the sites of action of two proteins. Ynl080c-GFP and Ynl081c-GFP fluorescence was visible in dot-shaped and elongated structures, whereas the Ynl022c-GFP signal was always found as one spot per cell, usually in the vicinity of nuclear DNA. The remaining C-terminal GFP fusions did not produce a clearly identifiable fluorescence signal. For 10 ORFs we constructed 5'-GFP fusions that were expressed from the regulatable GAL1 promoter. In all cases we observed GFP fluorescence upon induction but the localization of the fusion proteins remained difficult to determine. GFP-Ynl020c and GFP-Ynl034w strains grew only poorly on galactose, indicating a toxic effect of the overexpressed fusion proteins. In summary, we obtained a discernible GFP localization pattern in five of 20 strains investigated (25%). A deletion phenotype was observed in seven of 21 (33%) and an overexpression phenotype in two of 10 (20%) cases.

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