**Abstract**

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|  | Hub1 is an evolutionarily highly conserved unconventional ubiquitin-like protein that func- tions in pre-mRNA splicing by binding to splicing factors Snu66 and Prp5. We observed a yeast two hybrid (Y2H) interaction of S. pombe Hub1 with Kreb’s cycle enzyme Fumarase (Fum1). We found that this interaction is mediated through W47 amino acid, which was not present in S. cerevisiae Hub1 and hence failed to interact with Fum1. With further experi- ments, we found the splicing and adduct-inducing importance of this surface in S. pombe. Along with H48, it forms a solvent-exposed hydrophobic patch and is required for main- taining an increased Hub1 protein steady-state level. The introduction of this hydrophobic surface failed to complement Hub1 deletion at higher temperatures in S. cerevisiae and showed splicing defects. In this study, we found the organism-specific evolutionary selec- tion of Hub1 protein in S. cerevisiae by investigating the possible reasons for evolutionary elimination of this hydrophobic surface, most likely associated with significant intron loss during evolution. |