EXPRESSION OF HUMAN HYPOXIA INDUCIBLE FACTOR 1 IN YEAST

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Hypoxia inducible factor-1 (HIF-1) is a transcription factor composed of a constitutively expressed subunit (HIF-1β or ARNT) and a regulatory subunit (HIF-1α), which is continuously degraded under normoxia. HIF-1α is the key factor in cellular response to low oxygen tension. The molecular mechanisms that underlie the full activation of the limiting subunit (HIF-1α) are poorly understood. In this study we determined the viability of expressing both subunits of human HIF-1 in *Saccharomyces cerevisiae* as a model study. We show that both intact subunits were expressed in *S. cerevisiae* grown under normoxia. HIF-1a expressed in yeast cells grown under normoxia migrated similarly (on SDS-PAGE) to HIF-1a present in protein extracts of hypoxic HeLa cells. We did not observe an immunocomplex signal in the region of 104 kDa (corresponding to the dephosphorylated form of HIF-1α) in Western Blot experiments, indicating that all the expressed HIF-1α in yeast was phosphorylated, as it is in mammalian cells exposed to hypoxia. Additionally, we show that recombinant HIF-1α was able to form a heterodimer with HIF-1β and bind to the human erythropoietin HRE (hypoxia response element) motif, while such binding was not observed in extract of yeast cells co-expressing the full length HIF-1α and a truncated version of HIF-1β.