

Title: Regulation of *C. elegans* behavior in response to hypoxic stress downstream of EGL-9/HIF-1

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Hypoxia resulting from low O₂ levels is a critical stress signal experienced by animals, resulting in physiological changes at the cellular, tissue and organismal level. The hypoxia recognition and response pathway is controlled by the O₂ dependent prolyl hydroxylase EGL-9 and transcription factor HIF-1, and is evolutionarily conserved from worms to mammals. In normoxic conditions EGL-9 utilizes O₂ to hydroxylate HIF-1, hydroxylated HIF-1 is then poly-ubiquitinated and degraded; in hypoxic conditions (<1% O₂), EGL-9 lacks one of its substrates, resulting in stabilization of HIF-1. Increased activity of HIF-1 causes adult *C. elegans* hermaphrodites to retain eggs inside the uterus, resulting in an Egl phenotype. This phenotype is observed in both hypoxic conditions and *egl-9(if)* mutants.

We previously found that the observed egg-laying defects of *egl-9(if)* mutants are controlled downstream of HIF-1 through the activation of the cytochrome P450 enzyme CYP-36A1, and the subsequent deactivation of the nuclear hormone receptor NHR-46. However, epistasis analysis also suggests that at least one additional pathway functions downstream of EGL-9 to partially control egg laying independent of the CYP-36A1 pathway. Identification of these parallel pathways would further define the complex effects of hypoxia on behavior, and provide insights into the specific tissues that coordinate this response.

From a forward genetic screen, we have isolated multiple mutants that suppress the *egl-9* egg-laying defects, but do not affect the transcription of *T24B8.5*, a downstream target of the CYP-36A1 pathway; genes represented by these mutations likely act in a pathway parallel to CYP-36A1 to control egg laying. Through an additional screen, we have isolated multiple mutations that enhance the ability of *cyp-36A1(if)* to suppress the egg-laying defect of *egl-9(if)* mutants. The isolation of these mutants supports our hypothesis that egg laying is controlled downstream of EGL-9 in-part by a pathway parallel to CYP-36A1. Through further genetic analysis, we hope to identify the pathway(s) that these mutants define, providing us with novel regulators of the EGL-9/HIF-1 mediated hypoxia stress response, a major pathway in animal biology and human disease.