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예쁜꼬마선충의 HIF-1 비의존적 저산소 반응에 대한 연구

HIF-1-independent Hypoxia Response of *hsp-16.1* in *Caenorhabditis elegans*

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Abstract

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During normal development and disease state, cells and organisms encounter low oxygen environments, a condition that is termed hypoxia. To overcome cellular damage and death that may result from oxygen deprivation, cells respond by expressing numerous hypoxia response genes, many of which are controlled by the hypoxia-inducible factor-1 (HIF-1). Recently, the cellular responses to hypoxia in the HIF-1-indedepent manner have been reported, but little is known about their molecular mechanisms. The nematode *Caenorhabditis elegans* (*C. elegans*) is one of the most important model systems for studying hypoxia response. In this study, I have identified core regulators that mediate hypoxia response and have examined how the expression of a hypoxia responsive gene can be modulated in the HIF-1-independent manner in *C. elegans*.

To better understand the mechanisms of HIF-1-independent hypoxia response, I have characterized the molecular basis of the hypoxia response of the *hsp-16.1* gene in *C. elegans*. This gene has been shown to be induced under hypoxic conditions in *hif-1* mutant animals, which demonstrates that hypoxia response of *hsp-16.1* is independent of *hif-1*. The HIF-1-independent

hypoxia response of *hsp-16.1* requires a conserved DNA sequence (CAC(A/T)CT) at its promoter region. Using affinity purification followed by LC-MS/MS, HMG-1.2 has been identified as a protein that binds to the conserved region of *hsp-16.1* under hypoxic conditions.

To further characterize the functional role of HMG-1.2 in hypoxia response, I employed a method termed 'functional gene network'. With this, I screened most of *C. elegans* orthologs shared with other species for biological interaction with *hmg-1.2* such as gene expression, co-citation, protein-protein interaction, and genetic interaction. I identified candidate genes that might interact with *hmg-1.2*. To validate these predictions, I performed RNAi to disrupt genes which were predicted to interact with *hmg-1.2*. I identified the chromatin modifiers *isw-1* and *hda-1*, histone H4, and the NURF-1 chromatin remodeling factor as new components of the *hif-1*-independent hypoxia response. These results suggest that the modulation of nucleosome positioning at the *hsp-16.1* promoter may be important for the *hif-1*-independent hypoxia response mechanism. In addition, I found that calcium ions are necessary for the induction of *hsp-16.1* in hypoxic conditions, and that calcineurin acts independently of *hif-1* to modulate the cellular response to hypoxia.

While the HIF-1 mediated gene regulation under hypoxic conditions has already been extensively studied, very little is known on HIF-1 independent response to hypoxia. Here I present a novel mechanism of hypoxia response where chromatin modification and calcium homeostasis may act independently of HIF-1 in *C. elegans*.

Keywords: *C. elegans*, *hif-1*-independent hypoxia response, *hsp-16.1*, HMG-1.2, chromatin remodeling factors, calcium

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I. Introduction

1. *Caenorhabditis elegans* as a model system for studying the mechanisms of hypoxia response.

During normal development, as well as in many disease conditions such as cardiovascular disease or cancers involving solid tumors, cells are faced with the challenge of oxygen deprivation. In order to cope with low oxygen levels, a condition termed hypoxia, cells respond by dynamically changing the expression of specific hypoxia-responsive genes (Semenza, 1998, 2003; Weidemann and Johnson, 2008). Consequently, understanding the regulatory mechanisms can provide opportunities to develop new targets for drugs and insights of diseases.

Caenorhabditis elegans, a free-living soil nematode, has emerged as a powerful model system to understand how organisms adapt and survive to oxygen deprivation. As a model organism, *C. elegans* has several advantages. First, it is inexpensive to grow in laboratory conditions and easy to manipulate. It can be cultured on agar plates with bacterial food. *C. elegans* is also a fast reproducing organism. At 20 ℃ under standard laboratory condition, it has a 4 day generation time (Brenner, 1974). *C. elegans* can be of two sexes: self-fertilizing hermaphrodite or male. Hermaphrodites can provide large brood sizes: they lay approximately 300 embryos that have isogenic genetic background and the cell lineages are invariant (Brenner, 1974; Johnson, 2003). The large number of offspring allows for easier discrimination of mutant phenotypes at the level of individual cells. In addition, it is relatively easy to introduce DNA into *C. elegans* using microinjection techniques, in order to obtain transgenic animals (Mello et al., 1991). This experimental convenience and simplicity make *C. elegans* an ideal model system for genetic analyses. With its genome fully sequenced, Genome-wide analyses are possible in *C. elegans*. The effects of perturbing most genes *in vivo* can be investigated using forward and reverse genetics techniques such as mutant screening and double-stranded RNA interference (Timmons et al., 2001). Finally, most of genes and signaling pathways in *C. elegans* are evolutionally conserved, meaning that they are also found in other organisms, including mammals. In

fact, many signaling pathways were first discovered using the *C. elegans* model system, before their homologs were found in mammals. For instance, the discovery of genes that are essential in programed cell death, a critical mechanism to understand many pathologies, were pioneered in *C. elegans* (Horvitz, 2003). In the field of hypoxia research, *C. elegans* provided the genetic basis for understanding the role of the hypoxia-inducible factor (HIF-1), an important factor in cell adaptation to hypoxic conditions (Epstein et al., 2001). For all these reasons, *C. elegans* is a critical model organism in the study of cellular responses to hypoxia.

2. Hypoxia-inducible factor (HIF)

The hypoxia-inducible factor (HIF) is the major regulator of many of the cell's physiological responses to hypoxia. For this reason, its roles in the cellular response to changes in oxygen concentration have been extensively studied (Kim and Kaelin, 2003; Semenza, 2003; Semenza et al., 1991; Semenza and Wang, 1992). HIF is a transcription factor which binds to the hypoxic response elements (HREs) in the promoter regions of HIF-1 target genes, and activates the hundreds of genes involved in angiogenesis, apoptosis, metabolism, and cell division (Schofield and Ratcliffe, 2004; Semenza, 2010). In terms of structure, HIF is a heterodimeric protein composed of two subunits, HIF-1α and HIF-1β, which belong to the Per-Arnt-Sim (PAS) family of basic helix-loop-helix proteins (bHLH) (Bunn and Poyton, 1996). The β subunit of HIF-1 is also known as ARNT (aryl hydrocarbon receptor nuclear translocation) and it is constitutively expressed (Jiang et al., 1996; Wang et al., 1995). While HIF-1β can dimerize with a number of other transcription factors (Swanson and Bradfield, 1993), HIF-1α is a hypoxically responsive component: its stability is regulated by oxygen concentration. HIF-1α contains the bHLH, PAS, oxygen-dependent degradation (ODD) domain, as well as two trans-activation domains (N-TAD and C-TAD). When oxygen levels are high, conserved proline residues 402 and 564 within the ODD domain of HIF-1α are hydroxylated by oxygen-

dependent enzymes belonging to a family of 2-oxoglutarate-dependent dioxygenases (Bruick and McKnight, 2001; Epstein et al., 2001), allowing them to be recognized by the von Hippel-Lindau tumor suppressor protein (VHL) (Ivan et al., 2001; Jaakkola et al., 2001; Min et al., 2002). VHL is a component of an E3 ubiquitin ligase complex and targets the α subunit of HIF for polyubiquitination and proteasomal degradation (Maxwell et al., 1999). Another hydroxylase of HIF-1 is the factor inhibiting HIF-1 (FIH-1), which hydroxylates the asparagine residue 803 within the C-TAD of HIF-1α (Mahon et al., 2001). Under normoxia, FIH-1 suppresses HIF-1's activity either by preventing the interaction between HIF-1α and the coactivator proteins p300/CBP, or through the recruitment of histone deacetylases. (Lando et al., 2002; Mahon et al., 2001). In hypoxic conditions, HIF-1α stabilizes and interacts with p300/CBP (Ema et al., 1999). Full HIF-1 activation requires additional regulations, including post-translational modifications, nuclear localization, and transcriptional cofactor recruitment (Wenger, 2002). In *C. elegans*, HIF-1 participates not only in the adaptive response to hypoxia, but is also involved in a number of other biological processes, including stress responses, behavioral responses, neuronal development, and aging (Bretscher et al., 2008; Chang and Bargmann, 2008; Chen et al., 2009; Jiang et al., 2001; Pocock and Hobert, 2008; Treinin et al., 2003).

The HIF-1 hypoxia response pathway is evolutionarily conserved in species as diverse as yeast, nematodes and mammals. For instance, *hif-1* is the *C. elegans* ortholog of mammalian HIF-1α, and *C. elegans* HIF-1 binds to AHA-1, the ortholog of mammalian ARNT/HIF-1β. A proline hydroxylase of HIF-1α, EGL-9, was first identified in *C. elegans*. *egl-9* mutant animals show constitutive expression of HIF-1 (Epstein et al., 2001).

3. Hypoxia-induced behavioral changes in *C. elegans*

The soil nematode *C. elegans* often encounters hypoxic microenvironments in its natural

habitat. When this occurs, wild-type worms reduce their metabolic rate and are able to continue their development and reproduction. In contrast, *hif-1* mutant animals are viable under normal oxygen levels but cannot survive when oxygen levels fall below 1 % (Jiang et al., 2001; Van Voorhies and Ward, 2000).

In laboratory conditions, *C. elegans* has behavioral preference for oxygen concentrations ranging between 5 to 12%. The animals sense oxygen levels via the soluble GCY-35 protein, a homolog of the mammalian guanylate cyclase, which is expressed in URX, AQR, and PQR neurons (Gray et al., 2004). However, the oxygen concentration preference of *C. elegans* is influenced by the presence of bacterial food, as well as by genetic variation. For instance, in the presence of food, wild-type N2 animals do not display avoidance of hyperoxic conditions. Conversely, animals with low activity of NPR-1, that encodes for a G protein-coupled neuropeptide receptor, avoid high oxygen levels in the presence of food. This avoidance of hyperoxic condition triggers the animals to aggregate and accumulate on the thickest part of a bacterial lawn, where oxygen levels are lowest (de Bono and Bargmann, 1998; Gray et al., 2004). In *npr-1* animals, the avoidance of higher oxygen concentrations is regulated by *gcy-35* which acts upstream of cyclic guanosine monophosphate (cGMP)-gated channels TAX-2 and TAX-4 (Gray et al., 2004).

The pattern of avoidance and tolerance of hyperoxic conditions in *C. elegans* is dependent on the activation or repression of HIF-1 (remember that HIF-1 is activated/induced through prolonged exposure of *C. elegans* to hypoxic conditions). In conditions when *hif-1* is activated, such as when wild-type N2 worms are exposed to chronic hypoxia and *egl-9* mutants, worms display a change in behavior as they start to avoid hyperoxic condition in the presence of food. Conversely, in conditions of inactive *hif-1*, such as *hif-1* mutants and wild-type N2 worms under normoxia, the presence of food suppresses the avoidance of hyperoxia (Chang and Bargmann, 2008).

4. Hypoxia-induced changes in chromatin

Cellular adaptation to hypoxic stresses requires changes in the expression of a large number of genes. Since DNA is tightly packed as chromatin, a change in chromatin state is necessary for either activators or repressors to access the DNA and regulate gene expression (Kornberg and Lorch, 1999). Chromatin is known to be regulated in three different ways: histone posttranslational modification, nucleosome mobilization by ATP-dependent chromatin remodeling factors and variable associations with non-histone proteins (Kouzarides, 2007).

Under hypoxic conditions, cells produce ATP through anaerobic glycolysis, whereby pyruvate is transformed into lactate instead of acetyl-coenzyme A (acetyl-Co A) (Koukourakis et al., 2005). As a result, lower levels of acetyl-Co A might globally decrease histone acetylation levels. Many of the post-translational modifications that histones undergo in hypoxic conditions are thought to be involved in the HIF-1α regulatory pathway. More specifically, these histone modifications are suspected to affect the ability of HIF-1α to bind with promoter regions of the different hypoxia-regulated genes. For example, hypoxic conditions have been shown to increase histone H3K9 acetylation at the promoter of vascular endothelial growth factor (VEGF) (Jung et al., 2005), a gene that is regulated by HIF-1α. In the case of hypoxia-repressed gene regulation, global reduction in acetylated H3-K9 was observed at the promoter of hypoxia-repressed gene such as survivin and novel immunogenic protein 3 (NIP3) during hypoxia (Hoffman et al., 2002; Murai et al., 2005). mRNA levels of HDAC also remarkably increase under hypoxia (Islam and Mendelson, 2006). Furthermore, increase of di- and tri-methylated H3-K9 was observed at the hypoxia-repressed surfactin protein A (Sp-A) gene (Islam and Mendelson, 2006).

ATP-dependent chromatin remodeling factors utilize the energy of ATP hydrolysis to alter chromatin structure. The SWI/SNF chromatin remodeling complex increases HIF-1α mRNA level and transcriptional activity, and is important for hypoxia-induced cell cycle arrest (Kenneth et al.,

2009). The imitation switch-1 (ISWI) is gene encoding for a chromatin remodeling complex that belongs to the SNF2 helicase family. ISWI induces FIH and regulates hypoxia-induced cellular response by reduction of autophagy and increase of apoptosis in hypoxia (Melvin et al., 2011).

5. Roles of ISWI and NURF in hypoxia response

The ISWI (imitation switch-1) gene was first identified in *Drosophila* and is highly conserved through evolution, as it is also found in yeast (ISW2) and humans (SNF2H) for instance (Lazzaro and Picketts, 2001; Tsukiyama et al., 1995; Tsukiyama et al., 1999). The *Drosophila* ISWI is an ATPase subunit present in three different chromatin remodeling complexes: the ATP-utilizing chromatin assembly and remodeling factor (ACF), the chromatin accessibility complex (CHRAC) and the nucleosome remodeling factor (NURF). ACF and CHRAC are involved in nucleosome assembly and spacing to establish regular, ordered, nucleosomal arrays. Conversely, NURF is required for disruption of regular arrays of nucleosome and transcriptional regulation (Ito et al., 1997; Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997).

Drosophila NURF consists of ISWI, NURF301, NURF38 and NURF55 (Gdula et al., 1998; Martinez-Balbas et al., 1998). NURF is required for the expression of genes that are critical in *Drosophila* development, such as body patterning, wing development and germ stem cell development in the ovary (Badenhorst et al., 2002; Pepin et al., 2007).

The *C. elegans* ortholog of Drosophila ISWI, *isw-1* was identified as a suppressor of the synMuv (synthetic multivulva) phenotype. SynMuv genes are negative regulator of vulval development. *isw-1* and *nurf-1* promote the ectopic vulval fates of all synMuv mutant combination caused by activation of the RAS pathway, suggesting that *isw-1* and *nurf-1* are positive regulator of vulval cell fate (Andersen et al., 2006).

6. High mobility group proteins: a potential element of hypoxia response pathway.

High mobility group box (HMGB) proteins are a family of non-histone chromosomal proteins that are abundant in nuclei. Mammalian HMGB proteins contain two HMG box domains and a carboxyl-terminal acidic tail (Bustin and Reeves, 1996). It is known that HMGB proteins bind preferentially to distorted DNA structure (Bustin, 1999; Thomas and Travers, 2001). Although HMGB proteins bind to DNA with little sequence specificity, they are involved in many biological processes. In particular, they bind to nucleosomes, thereby loosening the wrapped DNA and enhancing its accessibility to chromatin remodeling complexes (Bonaldi et al., 2002; Bustin and Reeves, 1996). HMGB proteins have also long been suspected to accelerate sliding activities of chromatin remodelers and enhance the binding of chromatin remodeling factors to nucleosomal DNA (Bonaldi et al., 2002). Post-translational modifications of HMGB proteins influence their cellular activities. For example, phosphorylation affects their nuclear localization (Zhang and Wang, 2008). Furthermore, HMGB1 and HMGB2, two specific HMGB proteins, can be acetylated by coactivator CBP in their N-terminal region, which modifies their affinity to DNA (Pasheva et al., 2004; Ugrinova et al., 2001). Binding of calcium ions to the C-terminal domain of HMGB1 is also known to modulate its DNA-binding properties (Stros et al., 1990; Stros et al., 1994).

The *C. elegans* HMG-1.2 protein is a homolog of the human HMGB2, exhibiting about 50 % identity within the HMG boxes. In a recent study, Mabon et al. (2009) showed that HMG-1.2 was upregulated by hypoxia in *C. elegans*, and could thus be involved in resistance to hypoxic conditions. HMG-1.2 might also have site-specific sequence recognition abilities, for instance in Wnt signaling in specific developmental roles (Jiang and Sternberg 1999). The site specificity – or lack thereof – of HMG-1.2 is thought to be dependent on its position 7 amino acid, which is a hydrophobic residue in sequence-specific boxes, as opposed to a proline in non-sequence-specific boxes like HMG-1.1 (Read

et al., 1994). Thus, *C. elegans* HMG-1.2 is able of sequence-specific recognition and has more specialized functions than the human HMGB1.

7. Clues of a HIF-1-independent hypoxia response pathway

Oxygen levels lower than 0.3% are often found in advanced solid tumors. Under such hypoxic condition, tumorous cells are triggered to overexpress HIF-1α, which is a critical transcription factor in angiogenesis and will thus allow the growth of vascular cells that increase the immediate availability of oxygen (Semenza et al., 1991). More precisely, HIF-1α, is a key regulator of the vascular endothelial growth factor (VEGF), which is one of the most important angiogenic factors induced in tumors. The importance of HIF-1α in angiogenesis has also been demonstrated in HIF-1α mutant mice, which die during early embryogenesis with severe vascular defects (Iyer et al., 1998; Ryan et al., 1998). Because of the critical role that HIF-1α plays in angiogenesis, the inhibition of HIF-1α is considered to be a very promising therapeutic target of tumor angiogenesis.

Despite the importance of the HIF-1 factor in hypoxia response, a number of elements also suggest the existence of HIF-1-independent pathway in cell response to low oxygen levels. For example, HIF-1α deficiency in colon cancer was shown to reduce tumor growth and proliferation, yet the hypoxic induction of VEGF remained active (Mizukami et al., 2005). In another study, mutations in HIF-1 binding sites on the promoter of VEGF did not have the expected effect of impeding hypoxic induction of VEGF. The reason for that was shown to be the oncogenic K-ras gene, which is able to induce VEGF in a HIF-1-independent manner (Mizukami et al., 2004).

Despite these clues on the existence of a HIF-1-independent regulating mechanism, only few attempts have been made so far to elucidate the regulatory mechanism governing HIF-1-independent adaptations to low oxygen levels. In a study on the hypoxia-induced alteration of gene expression in *C.*

elegans hif-1 (ia04) mutants, Jiang et al. (2001) reported that HIF-1-independent pathways are involved in the adaptation to hypoxia. An earlier study also revealed that the response of small heat shock protein (HSP) *hsp-16* genes (*hsp-16.1* and *hsp-16.2*) to hypoxic conditions is HIF-1-independent, and occurs via *cis*-acting DNA sequences (CAC(A/T)CT), hereafter referred to as "block I", in the promoter region of these genes (Hong et al., 2004).

8. Roles of *hsp-16* in *C. elegans* under stress conditions

Many molecular chaperones are members of the heat shock proteins (HSP) family, and are highly expressed during cellular stresses in order to stabilize unfolded proteins and prevent the accumulation of aggregated proteins (Gething and Sambrook, 1992; Hendrick and Hartl, 1993). Whereas some chaperones including HSP40, HSP60, HSP70 mediate protein folding under normal conditions (Hartl, 1996; Langer et al., 1992), inducible HSP70s and small HSPs are involved in protecting organisms against stresses (Feder et al., 1996; Landry et al., 1989). In *C. elegans*, there are six 16 kD small HSPs that are members of α-crystallin, four of which have been extensively studied: *hsp-16.1*, *hsp-16.2*, *hsp-16.41* and *hsp-16.48* (Leroux et al., 1997; Russnak and Candido, 1985). These four HSP-encoding genes are similar in their structure and sequence homology (Candido et al., 1989). *hsp-16.1* and *hsp-16.48* are located in divergent orientations and separated by approximately 300 bp. An identical pair (*hsp-16.49*/*hsp-16.11*) is duplicated 400 bp away from *hsp-16.1*/*hsp-16.48*. *hsp-16.2* and *hsp-16.41* have the similar organization as *hsp-16.1*/*hsp-16.48*, except that they are not duplicated (Candido et al., 1989; Jones et al., 1986; Russnak and Candido, 1985). While HSP-16 proteins are not expressed under normal condition, they are highly induced by various stresses such as heat shock, reactive oxygen species, β-amyloid peptide, heavy metals, and oxidative stress (Jones et al., 1989; Stringham et al., 1992; Yanase et al., 1999). Therefore, HSP-16 proteins are thought to be a contributing factor in cell stress resistance. In this regard, it is interesting to note that HSP-16 was

found to affects lifespan, most likely through decrease of oxidative stress, interaction with nuclear hormone receptors and prevention of protein aggregation (Walker and Lithgow, 2003). A recent study also showed that induction of HSP-16.1 through preconditioning could protect *C. elegans* against heat-induced necrosis: animals that were pre-exposed to mild heat stress became more resistant against heat-induced necrosis than non-preconditioned animals (Kourtis et al., 2012).

9. Objective of this study

Here I aim to elucidate the alternative hypoxia response pathway which does not rely on HIF-1 function. To establish the HIF-1-independent hypoxia response pathway, I dissected the molecular mechanism that mediates the hypoxia-inducible transcription of the *C. elegans hsp-16.1* gene. I identified a potential factor that is involved in HIF-1-independent hypoxia response of *hsp-16.1*, using affinity chromatography purification followed by LC-MS/MS. To further characterize the mechanisms of HIF-1 independent hypoxia response, I employed an interactome network approach, which reveals direct and indirect interactions based on yeast, worm, fly and human orthology. Thereafter, I experimentally validated whether these genes are involved in the hypoxia response of *hsp-16.1* expression. These results indicate that chromatin remodeling complex proteins are involved in the modulation of the *hsp-16.1* expression under hypoxic conditions. I also show that this hypoxia-inducible transcriptional regulation is mediated by calcium signaling. Taken together, my results demonstrate the existence of an alternative, HIF-1-independent, mechanism by which cells adapt to hypoxic conditions.

II. Materials and Methods

Worm strains and culture

The standard protocol for maintaining *C. elegans* strains was used as described previously (Brenner, 1974). *C. elegans* strains were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN, USA). N2 strain was used as wild-type worms.

GFP fusion constructs and microinjection

The full-length *hsp-16.1::gfp* fusion plasmid was constructed by subcloning the full-length PCR product from the genomic T27E4.8 sequence into pPD95.77 (a gift of A. Fire, Stanford, CA). To generate the *hmg-1.2::gfp* construct, DNA was amplified by PCR. The PCR product included the 2 kb region upstream of the F47D12.4 promoter and the whole genomic sequence was cloned into pPD95.77. Microinjection was carried out using standard procedures. The pRF4 plasmid containing the dominant mutant *rol-6* gene was used as a marker. The *hsp-16.1::gfp* transgene was integrated into the genome by UV irradiation using Spectrolinker XL-1000 (Spectronics, Rochester, NY).

RNA interference assay

Baterial feeding protocol was used in RNAi experiment as described previously (Kamath et al., 2003; Timmons et al., 2001). HT115 bacteria carrying L4440, the plasmid of the empty vector pPD129.36, were used as a control. Most feeding RNAi clones were obtained from the Ahringer RNAi library (Geneservice, Cambridge, UK). Feeding vectors containing Y53F4B.3 were obtained from Vidal feeding RNAi library (Open Biosystems, Huntsville, AL). For *nurf-1*, a 950 bp PCR

fragment of cDNA corresponding to nucleotides spanning 251-1201 of F26H11.2 was subcloned into pPD129.36 (a gift of A. Fire, Stanford, CA). For H20J04.2, a 900 bp sequence of the third exon was amplified by PCR and subcloned into pPD129.36. In most experiments, we placed L4 stage worms (parents or P0) on a plate seeded with a bacterial strain carrying specific RNAi plasmids. For *hda-1*, which is an essential gene that causes lethality when severely knocked down, we placed synchronized L1 stage worms under RNAi condition and performed a hypoxia assay 3 days later.

Heat shock and hypoxia assay

The heat shock treatment was carried out at 30°C for 6 hours. For hypoxia treatment, we soaked worms in M9 buffer without rocking, as described by Hong et al. (2004). The worms were allowed to recover on NGM media at 20°C before analysis.

Chromatin immunoprecipitation (ChIP)

The ChIP experiments were adapted from Oh et al. (2006) and were performed using a ChIP assay kit (Upstate). Mixed-stage HMG-1.2::GFP transgenic worms were grown on NGM-lite plates and then harvested. Control worms were rocking while hypoxia worms were soaked in M9 without shaking. The worms were fixed in M9 buffer containing 2% formaldehyde at room temperature for 30 min. The reaction was quenched with 2.5 M glycine and washed with M9 buffer three times. Lysates were prepared as described above. The lysates were precleaned with salmon sperm DNA/protein G agarose beads and incubated overnight at 4°C with either anti-GFP antibody or IgG. The precipitates were washed, the crosslinks were reversed, and the DNA was eluted. Real-time quantitative PCR was

performed using a BioRad iQ SYBR Green Supermix in a BioRad IQ5 Real-Time PCR machine. The following primer sets were used: sense 5'-AGGTGCAAAGAGACGCAGAT-3' and antisense 5'-CTAGAACATTCGAGCTGCTT-3'.

Microscopy and measurement of fluorescence intensity

All images were taken using an Axioplan2 microscope equipped with an AxioCam HRc and Axiovioion 4.7 software (all Zeiss). Fluorescence intensity was analyzed using ImageJ software by outlining the second intestinal cells of the worms. The density was normalized to the L4440 control. Either a one-way ANOVA ($p<0.05$) or an unpaired t-test were employed to find genes that were significantly different between control and RNAi at each time. All of the data are expressed as the mean ± SEM.

Nucleosome preparation and micrococcal nuclease (MNase) assay

Mixed-stage worms were pelleted and frozen in buffer A [250 mM sucrose, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EGTA, 0.2 mM PMSF, and 7 mM β–mercaptoethanol] and protease inhibitor set III (CalBiochem). The worms were ground into a fine powder in liquid nitrogen. The resultant worm dust was resuspended in buffer A. Micrococcal nuclease (MNase; Roche), resuspended at 300 U/μL in 10 mM Tris-HCl (pH 7.4), 15 mM NaCl, 60 mM KCl, 0.15 mM spermine and 0.5 mM spermidine, was added to the worm extract. Digestions were performed at 25°C and stopped by adding worm lysis buffer [0.1 M Tris-HCl (pH 8.5), 0.1 M NaCl, 50 mM EDTA, and 1%

SDS], and the sample was treated with proteinase K (20 mg/mL) for 45 min at 65°C. Purified DNA without MNase treatment was digested with *Bgl*II (Enzynomics), and one of the enzymes *Pst*I, *Rsa*I (Fermentas), or *Xba*I (Roche). The same amount of DNA was loaded onto a 1.4% agarose gel, and the separated DNAs were transferred to a nylon membrane. The 200 nt fragment of DNA close to the *Bgl*II site within the *hsp-16.48* gene as indicated in Figure 15 was ^{32}P-labeled using random prime labeling kit (Amersham) for indirect end-label analysis.

EGTA and thapsigargin treatment

Worms were treated with EGTA under hypoxic and heat shock conditions in different concentrations (0, 100, 200, 400 mM). Thapsigargin (Sigma) was treated at a final concentration of 1.25 μM while rocking for 6 hours. As a control experiment, the same amount of DMSO was treated in parallel.

Streptavidin purification and protein identification

Biotin-labeled oligonucleotides were synthesized (Bionics, Seoul, Korea). The biotin-labeled DNA consisted of either 38-base pair oligonucleotides containing block I sequences or sequences in which block I sequences had been replaced with TTTTTT. Based on previous findings, which indicated that base substitutions dramatically reduced competitive activity (Hong et al. 2004), T-substituted oligonucleotides were considered suitable for excluding proteins that bound in a non-specific manner. To immobilize the biotinylated oligonucleotides on streptavidin sepharose (GE Healthcare), 6 μmol of the oligonucleotides was incubated with streptavidin sepharose for 2 hours.

Nuclear extracts from the worms under hypoxic conditions were loaded on the immobilized sepharose column in affinity buffer [10 mM HEPES (pH 7.6), 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 5% glycerol, and 0.025% NP-40]. After extensive washing, proteins were eluted in 20 mM HEPES, 1 mM EDTA, and 2 M NaCl. All of the buffers contained protease inhibitor set III (CalBiochem). The eluate followed by TCA precipitation was loaded onto an SDS-PAGE gel (Novex). The gel was stained with Coomassie blue, and the bands were excised from the gel. The resultant bands were analyzed by MALDI-TOF and quadrupole Time-of-flight (Q-TOF) mass spectrometry (In2Gen, Seoul, South Korea). The sequences of the oligonucleotides used were as follows: block I (5'-CTGGGTTTCTTCAGTACGCACACTATTTCTCAATGTTC-3'); and T substituted block I (5'-CTGGGTTTCTTCAGTACGTTTTTTATTTCTCAATGTTC-3').

Database searches and sequence alignment

We sequenced the DNA of the *his-26*, *his-38*, *his-64* and *his-67* RNAi clones and conducted a BLASTN search (WormBase release WS 229). Sequence alignment of the histone H4 genes was performed using MacVector 11.0.2 software

III. Results

1. Identification of HMG-1.2 as a protein that binds to CAC(A/T)CT motif

A Previous study showed that the sequence CAC(A/T)CT (block I) was required for the HIF-1-independent hypoxia response of *hsp-16* genes and also demonstrated proteins bound to block I-containing sequences in vitro (Hong et al., 2004). To identify the regulatory proteins that bind the *hsp-16.1* promoter in hypoxic conditions, I isolated nuclear extracts from worms incubated in hypoxic conditions and applied them to streptavidin affinity columns containing biotin-labeled block I-containing sequences. As a negative control, I used a biotin-labeled probe in which the block I sequences were mutated (Fig. 1). I analyzed the isolated proteins using MALDI-TOF and quadrupole Time-of-flight (Q-TOF) mass spectrometry (Fig. 2). By comparing the results from the block I-containing column to those from the block I-mutated column, I excluded non-specific binding proteins, including HMG-1.1. This analysis showed that HMG-1.2 was the predominant protein that was bound specifically to the block I-containing sequence. I then performed chromatin immunoprecipitation (ChIP) to confirm whether HMG-1.2 was bound to the block I-containing promoter in vivo and whether the binding efficiency of HMG-1.2 was affected by hypoxia. I found that HMG-1.2 binding was enriched more than twenty fold under hypoxic conditions compared to normal conditions (Fig. 3). Taken together, my results suggest that HMG-1.2 proteins specifically bind to the block I element of the *hsp-16.1* promoter in response to hypoxic conditions.

2. Role of HMG-1.2 in hypoxia response of *hsp-16.1*

$P_{hmg-1.2}$::HMG-1.2::GFP localizes in most somatic cells in the nuclei and hypoxia affects neither its expression level nor localization (Fig. 4 and Fig. 5). To investigate the potential roles of HMG-1.2 in the HIF-1-independent response to hypoxia, I performed RNA interference (RNAi) to reduce

HMG-1.2 activity. L4 larvae were fed double-stranded RNA (dsRNA)-producing bacteria. Thereafter, I monitored the expression of *hsp-16.1* fused with GFP (HSP-16.1::GFP) in F1 progeny exposed to hypoxic conditions. I also determined whether the expression of *hsp-16.1* was regulated in a hypoxia-specific manner or as a general response to stress. To do so, worms were also incubated under heat shock conditions, as heat shock can induce *hsp-16.1* gene expression (Jones et al., 1989; Stringham et al., 1992). Under hypoxic conditions, disruption of *hmg-1.2* activity by RNAi, when compared to the empty vector control, led to an approximately 60% decrease in the level of HSP-16.1::GFP fusion protein. On the other hand, *hmg-1.2* RNAi did not affect the induction of HSP-16.1 in response to heat shock treatment (Fig. 6A and B). These observations suggest that induction of *hsp-16.1* by hypoxia, but not by heat shock, specifically required functional HMG-1.2. As a negative control, I observed no significant change in HSP-16.1::GFP expression in response to either stress in the *hmg-1.1* RNAi animals.

I also examined the effect of *hmg-1.2* inactivation by RNAi on the activation of *hsp-16.1* in the *hif-1 (ia04)* background. I found that *hsp-16.1* was strongly induced in *hif-1 (ia04)* mutants and that the induction of *hsp-16.1* was decreased by *hmg-1.2* RNAi but not by *hmg-1.1* RNAi in these mutant animals. Importantly, these observations were similar to those in wild type animals (Fig. 7A and B). Induction of *hsp-16.1* by heat shock was not affected in *hif-1 (ia04)* mutants. These results are consistent with the notion that the hypoxia response of *hsp-16.1* is not mediated by the *hif-1* pathway.

3. Hypoxia-inducible expression of *hsp-16.1* through chromatin remodeling factors and histone 4

It has been suggested that if two genes are connected in any way, they are likely to participate

in a common biological process (Von Stetina and Mango, 2008). Therefore, I used functional gene networks to further characterize the mechanism by which *hmg-1.2* mediates the HIF-1-independent hypoxia response. I used tools provided by the WormNet website (http://www.functionalnet.org/wormnet/) (Lee et al., 2008; Lee et al., 2010) that can predict the genetic or physical interaction of a gene with a gene of interest, in my case *hmg-1.2*. Wormnet provides functional gene networks by combining different types of data such as co-cited gene associations, mRNA coexpression, protein-protein interaction, physical interaction and genetic interaction among *C. elegans*, yeast, fly, and human proteins. Probability of interactions is scored with a Bayesian network (Lee et al., 2008; Lee et al., 2010; Lee et al., 2007a; Lehner and Lee, 2008). Genes predicted to interact with *hmg-1.2* included a transcription factor (*mab-5*), a nuclear transport factor, a protein kinase (*hpk-1*) and several chromatin-associated genes such as a predicted subunit of FACT (facilitates chromatin transcription) (*hmg-4*), components of the chromatin remodeling complex (*psa-4* and *isw-1*), histone H4 coding genes, an H2A.Z histone variant (*htz-1*) and histone deacetylase (*hda-1*) (Fig. 8 and Table 1). I then inactivated the candidate genes using RNAi to test whether they were involved in the hypoxia response of *hsp-16.1*. While I found no effect for some genes, such as *mab-5*, *ima-3*, *hpk-1*, *hmg-4*, *psa-4*, and *htz-1*, I did find that the inhibition of *his-26*, *his-38*, *his-64*, *his-67*, *isw-1*, and *hda-1* by RNAi decreased the expression of the *hsp-16.1* gene under hypoxic conditions (Fig. 9A and B). Given the sequence similarity between *his-26, his-38, his-64* and *his-67* and with their similarity to other histone H4 genes (Fig. 10), it is conceivable that the inactivation of the four histone H4 genes by RNAi may also have resulted in the silencing of other histone H4 genes. ISW-1 is an ortholog of the Drosophila ISWI and acts as an ATPase in chromatin remodeling complexes. HDA-1 is a histone deacetylase involved in chromatin modification. My results thus suggest that chromatin modifiers are involved in the hypoxia response of *hsp-16.1*. Furthermore, the inactivation of *isw-1* and histone H4 coding genes by RNAi in *hif-1 (ia04)* deletion mutants showed results that were consistent with the HIF-1 independence of *hsp-16.1* in wild type worms (Fig. 11A and B).

4. NURF chromatin remodeling complex may mediate the *hif-1*-independent hypoxia response of *hsp-16.1*

There are three chromatin-remodeling complexes that contain the ATPase subunit ISWI: ATP-dependent chromatin assembly and remodeling factor (ACF) (Ito et al., 1997), chromatin accessibility complex (CHRAC) (Varga-Weisz et al., 1997) and nucleosome remodeling factor (NURF) (Tsukiyama and Wu, 1995). Genes encoding these components of chromatin remodeling complexes are well conserved in *C. elegans*: ACF1 (*flt-1* and *athp-2*), CHRAC-14 (T27A5.8) and CHRAC-16 (Y53F4B.3) (Andersen et al., 2006). In the Drosophila NURF complex, ISWI is composed of NURF301, NURF38 and NURF55 (Gdula et al., 1998; Martinez-Balbas et al., 1998); these subunits are homologous to *nurf-1*, *pyp-1* and *rba-1* in *C. elegans*, respectively. I determined whether the *C. elegans* orthologs of the ACF, CHRAC, and NURF proteins were required for the hypoxia response of *hsp-16.1*, similar to *isw-1*. Inactivation of *flt-1*, *athp-2*, T7A5.8 and Y53F4B.3 did not affect the induction of *hsp-16.1* in response to hypoxia (Fig. 12). Only RNAi inhibition of the gene encoding the NURF301 ortholog failed to fully induce *hsp-16.1* by hypoxia but not by heat shock (Fig. 13A and B). Furthermore, I found that NURF-1 regulation of *hsp-16.1* is independent of HIF-1 (Fig. 14A and B). My results indicate that ISW-1 likely functions as a component of the NURF complex to regulate the expression of *hsp-16.1* in response to hypoxia and that this activity is independent of HIF-1.

5. Hypoxia response occurs through the modification of chromatin environment

Next, I investigated the roles of ISW1 and NURF during hypoxia. Because NURF-1 has been shown to have nucleosome disruption activity in vitro (Mizuguchi et al., 1997; Xiao et al., 2001) and because the chromatin structure of *hsp-16* genes has been shown to markedly differ upon heat shock

treatment (Dixon et al., 1990), I decided to examine whether hypoxia affected the chromatin structure of the *hsp-16.1* promoter in vivo. I purified nuclei from control and hypoxia-treated worms and subsequently performed MNase digestion coupled with Southern blot analysis to map nucleosome positioning. Purified nuclei were free from endonucleases as no degraded bands were observed (Fig. 15, lanes 1, 2). The differences in nucleosome arrays were obvious when nuclei were partially digested by MNase (lanes 3, 4, 5, 6). To map the relative positions, I digested naked DNA with restricted enzymes (data not shown). An MNase hypersensitive site was detected in normoxia condition near *Pst*I and *Xba*I sites, which marks the HSE (asterisk, lanes 3, 5, 7), and this was attenuated in hypoxia (lane 4, 8). In contrast, an MNase sensitive site appeared in hypoxia-stressed nuclei (open circle, lanes 4, 6), which corresponds to the block I region. These data suggest that nucleosome remodeling has occurred along the block I region in response to hypoxia and that the block I region is exposed outside of nucleosomes, which in turn results in the transcriptional activation of *hsp-16.1*.

6. Calcium signaling may mediate the hypoxia response in a HIF-1-independent manner

Cells are known to increase intracellular calcium levels as a primary response to hypoxia (Buckler and Vaughan-Jones, 1994). Furthermore, the binding of calcium to the C-terminal domain of HMGB1, another mammalian homolog of HMG-1.2, is known to modulate HMGB1's DNA-binding properties (Stros et al., 1990; Stros et al., 1994). Therefore, I investigated whether the disruption of the cellular calcium balance affects the hypoxia response of *hsp-16.1*. I used chemical reagents to manipulate the endoplasmic reticulum's release of calcium: thapsigargin, which induces the release of endoplasmic reticulum calcium, and EGTA, which specifically chelates calcium (Bandyopadhyay et

al., 2002). I found that treatment with EGTA decreased the induction of *hsp-16.1* in response to hypoxia in a concentration-dependent manner, whereas heat shock responsive activation remained unaffected (Fig. 16A and B). In contrast, thapsigargin treatment was sufficient to activate *hsp-16.1* under normal oxygen concentrations (Fig. 17). Interestingly, *tax-6*, which encodes the catalytic subunit of calcineurin, was up-regulated in the *hif-1 (ia04)* mutant animals under hypoxic conditions (Shen et al., 2005). Calcineurin contains a catalytic subunit, calcineurin A, and a regulatory subunit, calcineurin B, which are encoded by the *C. elegans* genes *tax-6* and *cnb-1*, respectively (Bandyopadhyay et al., 2002). I found that inactivating *tax-6* or *cnb-1* resulted in failure to induce *hsp-16.1* expression under hypoxia (Fig. 18A and B). This phenotype is similar to that of *hif-1 (ia04)* mutants (Fig. 14A and B). Taken together, my results indicate that changes in cellular calcium levels generate a primary signal that induces the expression of *hsp-16.1* in response to hypoxia.

7. C12C8.1 gene might require HMG-1.2 for its response to hypoxia

As the *hif-1*-independent hypoxia response regulatory mechanism might not act solely on *hsp-16.1*, I examined 47 candidate genes that could potentially also be regulated through this mechanism. These 47 genes were originally isolated through whole genome microarray technique (Shen et al., 2005). Using real-time quantitative PCR, I examined whether the expression of these genes is affected by hypoxia, and whether it is mediated by HMG-1.2. Only mRNA levels of C12C8.1 *(hsp-70)* and F44E5.4 were exclusively induced by hypoxia (Fig. 19). The promoter in the block I region of C12C8.1 shares great similarity with the promoter of *hsp-16.1*, but not with the one of F44E5.4 (Fig. 20). Although the mRNA level of C12C8.1 was not affected by *hmg-1.2* RNAi, its protein level was diminished by *hmg-1.2* RNAi under hypoxia (Fig. 21A). The block I region is also required for the hypoxia response of C12C8.1 as well as that of *hsp-16.1* (Fig. 21B). While under normal oxygen

concentrations, C12C8.1 is expressed only in the ventral cord of *C. elegans*, it becomes highly upregulated in the intestine, body wall muscle cells, and vulval muscle cells when the animals are exposed to hypoxic conditions (Fig. 22).

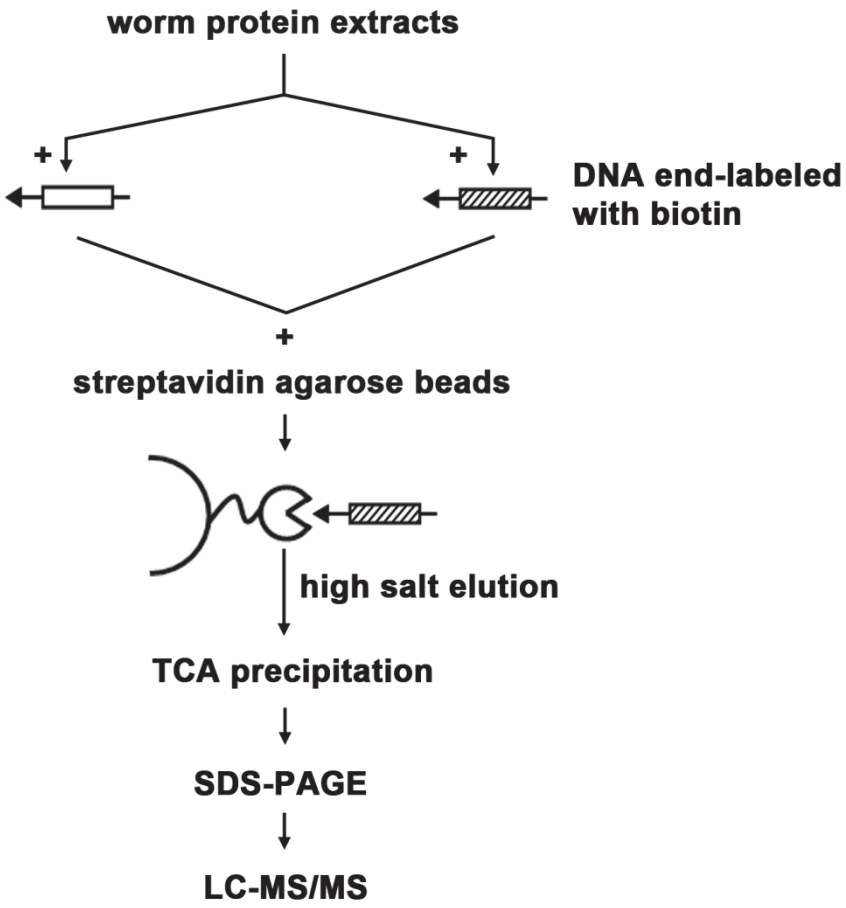
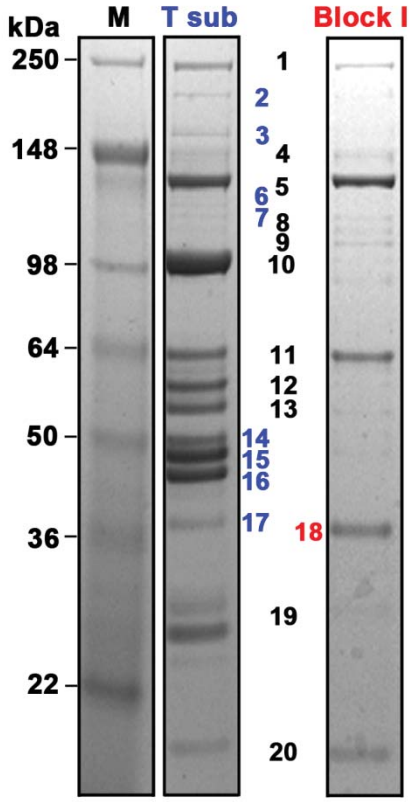


Figure 1. Schematic diagram of the purification of block I-binding proteins.

Worms for the protein extracts were prepared under hypoxic conditions. The dashed boxes represent block I (CAC(A/T)CT), and the blank boxes represent TTTTTT nucleotides substituted for block I as a control experiment. The proteins immobilized to the biotin-labeled DNA and streptavidin agarose were eluted, followed by TCA precipitation. After protein separation by SDS-PAGE, each protein band was sequenced using mass spectrometry (LC-MS/MS).



No.	Gene	description
1	*unc-54*	myosin class II heavy chain unc-54
2	*vit-4*	vitellogenin structural genes vit-4
3	*msh-6*	msh(muts homolog) family protein 6 msh-6
4	K07H8.10	AAA ATPase containing von Willebrand factor type A (vWA) domain
5	*pme-1*	poly ADP-ribose metabolism enzyme-1
6	B0507.8	novel protein
7	*hus-1*	ortholog of the Schizosaccharomyces pombe Hus1 DNA damage checkpoint protein
8	*cku-80*	*ortholog of human XRCC5 (Ku80)*
9	*cku-70*	*ortholog of human XRCC6 (Ku70)*
10	*rpa-1*	replication protein A(RPA) large subunit (70kD)
11	*vig-1*	RNA-binding protein orthologous to Drosophila VIG
12	F21D5.5	polynucleotide kinase 3' phosphatase
13	*tiar-1*	apoptosis-promoting RNA-binding protein TIA-1/TIAR
14	*rnp-1*	RNA-binding protein LARK, contains RRM and retroviral-type Zn-finger domains
15	*sqd-1*	homologous to drosophila sqd protein 1
16	*rpa-2*	medium subunit of replication protein A (RPA)
17	*sqd-1*	homologous to drosophila sqd protein 1
18	*hmg-1.2*	*HMG box-containing protein*
19	*hmg-11*	hmg-I-alpha
20	*hmg-1.1*	*high mobility group (HMG) family of proteins*

Figure 2. Identification of block I-binding proteins.

Purified proteins were separated on DSD-PAGE gels and stained with Coomassie Blue. The identities of the bands were confirmed by MALDI-TOF and quadrupole Time-of-flight (Q-TOF) mass spectrometry. Molecular mass standards (kDa) are shown on the left. HMG-1.2 bound specifically to block I. Red indicates the proteins purified from block I. Blue indicates the proteins purified from the mutated probe. Black represents the proteins purified from both columns.

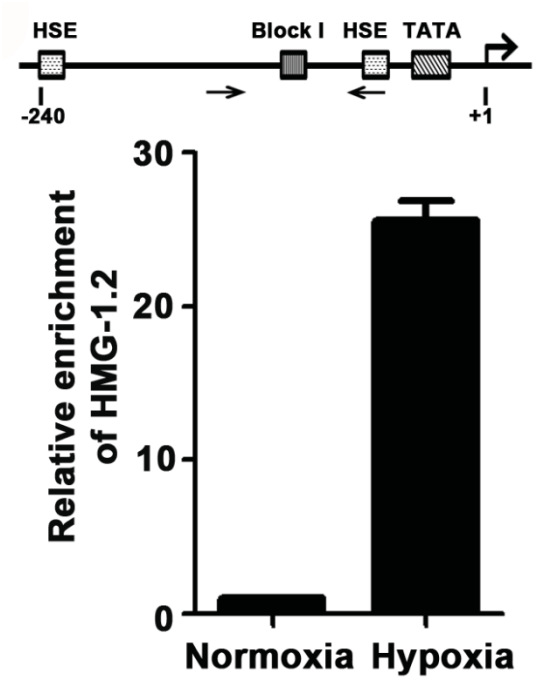


Figure 3. HMG-1.2 binds to block I region *in vivo*

Chromatin immunoprecipitation (ChIP) with anti-GFP antibody and real-time PCR (qPCR) revealed that HMG-1.2 is directly associated with block I under hypoxic conditions. HSE: heat shock response element, TATA: TATA box. The arrows indicate the primers used for qPCR

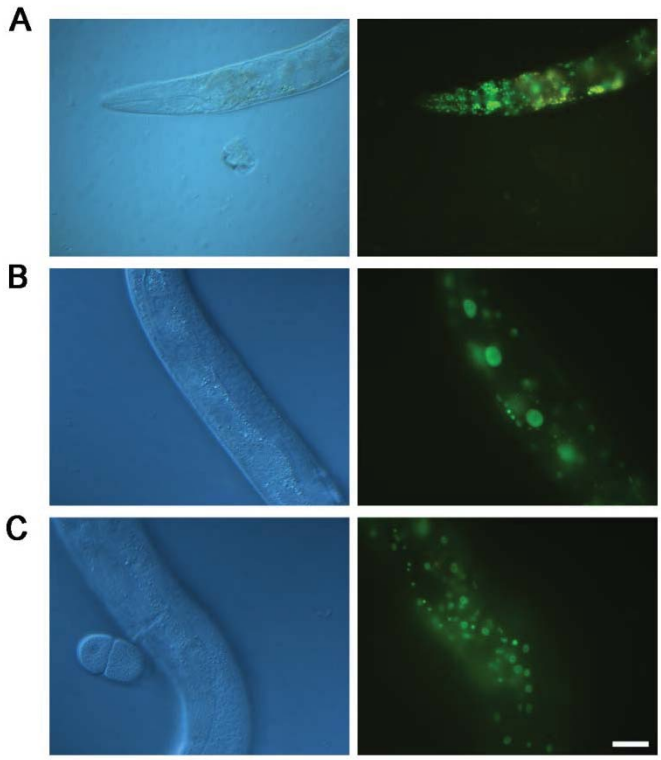


Figure 4. Expression pattern of HMG-1.2::GFP in *C. elegans*.

HMG-1.2 is expressed in most, if not all, somatic cells in the nuclei **(A)** Unidentified head neurons. **(B)** Intestine. **(C)** Hypodermis. The scale bar represents 20 μm.

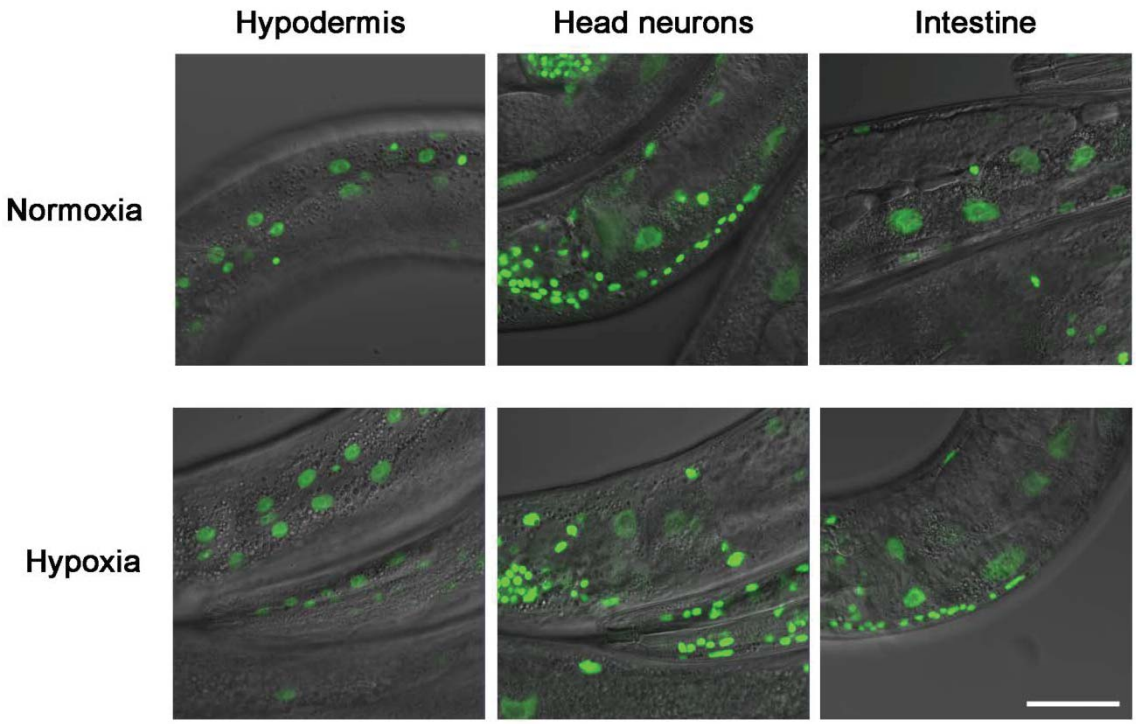


Figure 5. Localization and the intensity of HMG-1.2 are not affected by hypoxia.

HMG-1.2 is expressed in hypodermis, head neurons and intestine and its sub-localization in nuclei is not altered by hypoxia. The scale bar represents 30 μm.

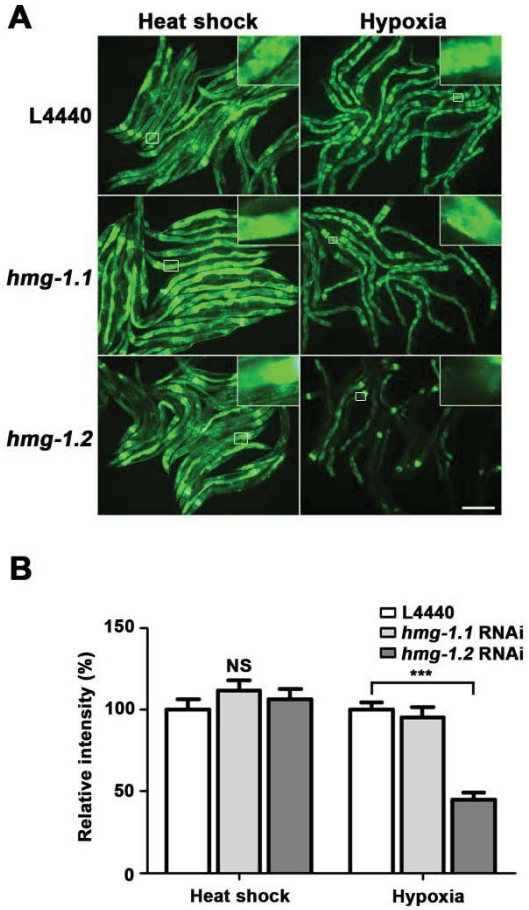


Figure 6. HMG-1.2 is a potential regulator of the *hsp-16.1* hypoxia response

(A) A reduction in *hmg-1.2* function significantly downregulates the induction of *hsp-16.1* under hypoxia, whereas *hmg-1.1* RNAi does not affect the *hsp-16.1* hypoxia response. Inlets are the second intestine cells which show the most consistent fluorescence intensity within worms. All of the photographs were taken under identical exposure conditions. The scale bar represents 200 μm. **(B)** The relative fluorescence intensity of stress-induced HSP-16.1::GFP was quantified using ImageJ software. Statistical significance was determined using a one-way ANOVA and Dunnett′s multiple comparison with the hypoxia controls (*** p 〈0.001). NS, not significant. The error bars indicate the SEM.

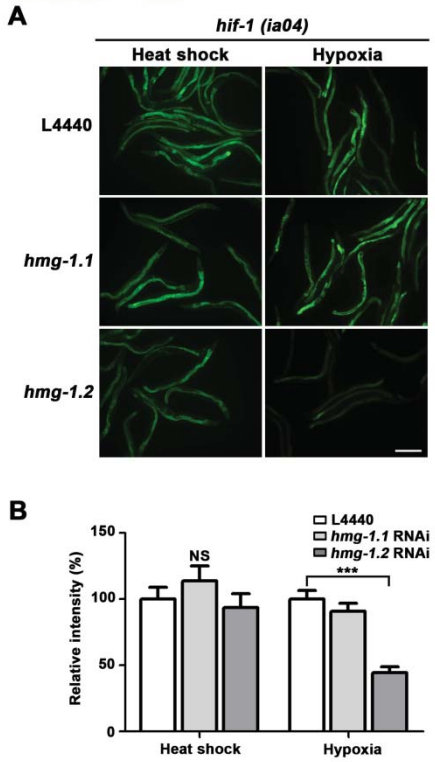


Figure 7. HIF-1 is not involved in the hypoxic regulation of *hsp-16.1* via HMG-1.2.

The effect of *hmg-1.2* inactivation on the activation of *hsp-16.1* in the *hif-1 (ia04)* background. **(A)** The hypoxia response of *hif-1(ia04)* mutant animals treated with *hmg-1.2* RNAi was similar to that observed in wild type animals. The levels of heat shock-induced *hsp-16*.1 were not altered by reduction in the function of *hmg-1.2* in *hif-1(ia04)* mutants, either. All of the photographs were taken under identical exposure conditions. The scale bar represents 200 μm. **(B)** The quantification data. The relative fluorescence intensity of stress-induced HSP-16.1::GFP was quantified using ImageJ software. Statistical significance was determined using a one-way ANOVA (Dunnett test. *** $p <$ 0.001). NS, not significant. The error bars indicate the SEM.

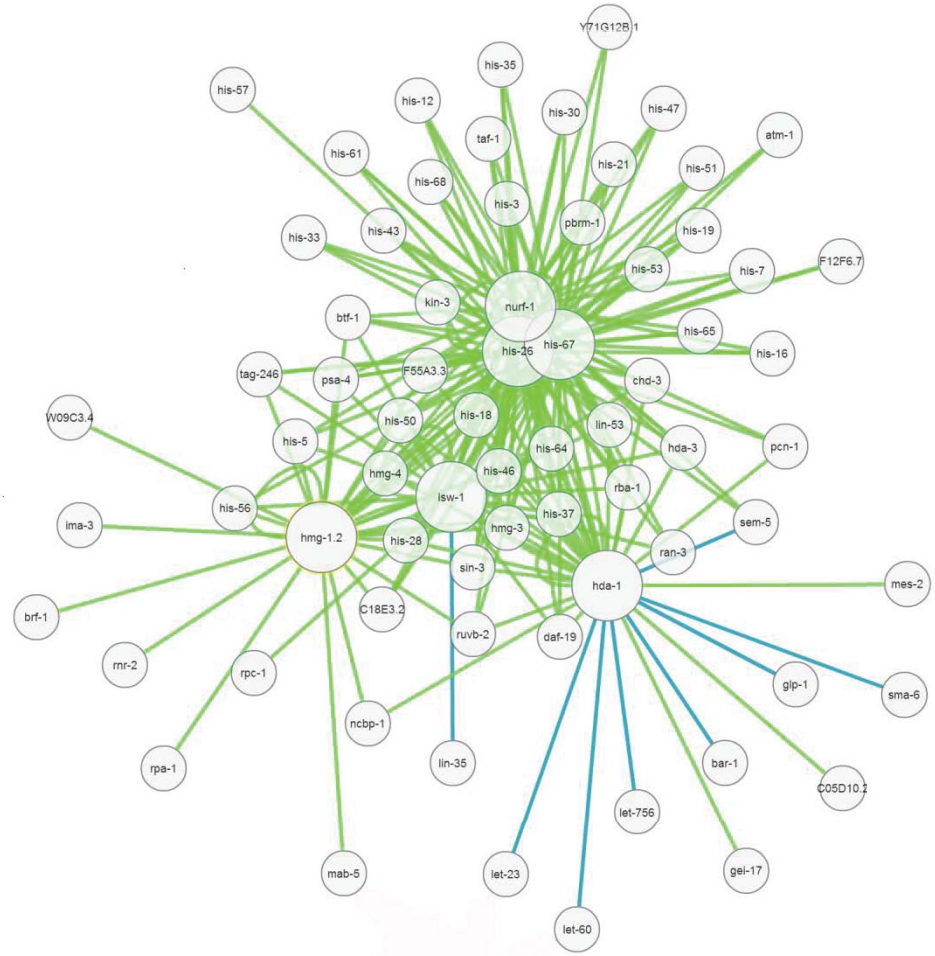


Figure 8. Prediction of potential interactions with HMG-1.2.

High network connectivity among the genes suggests high probability of interactions. Experimentally proven physical interactions are shown as blue lines.

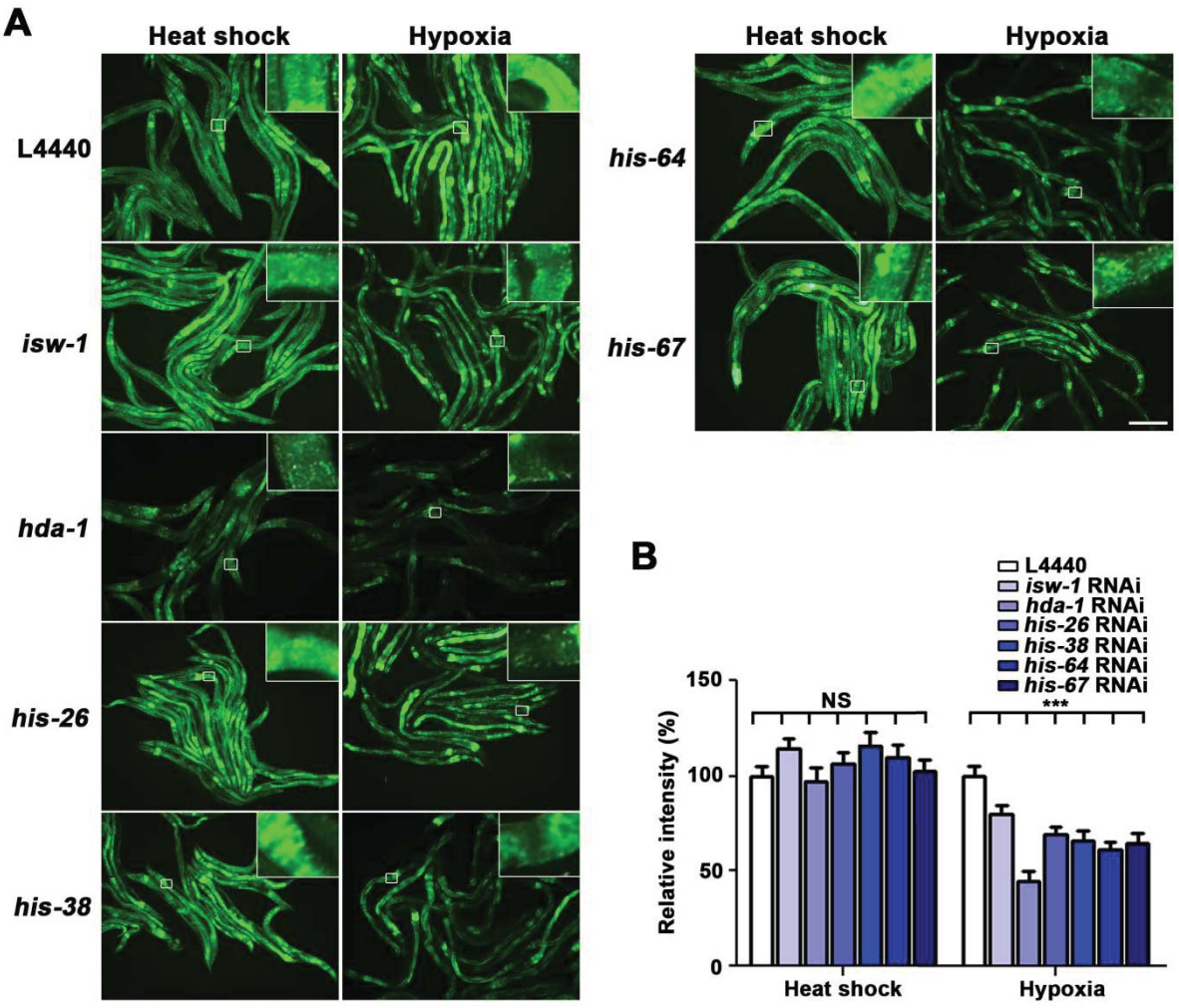


Figure 9. Chromatin remodeling components, histone deacetylase and histone H4 are involved in *hsp-16.1* hypoxia response.

(A) A reduction in *isw-1*, *hda-1*, *his-26*, *his-38*, *his-64* and *his-67* RNAi suppresses the hypoxia upregulation of *hsp-16.1*. The scale bar represents 200 μm. **(B)** The quantification of relative intensity of HSP-16.1::GFP fluorescence was carried out using ImageJ software. The triple asterisks indicate values that differ from the L4440 hypoxia controls a the p ⟨0.001 significance level (Dunnett test). NS, not significant. The error bars indicate the SEM.

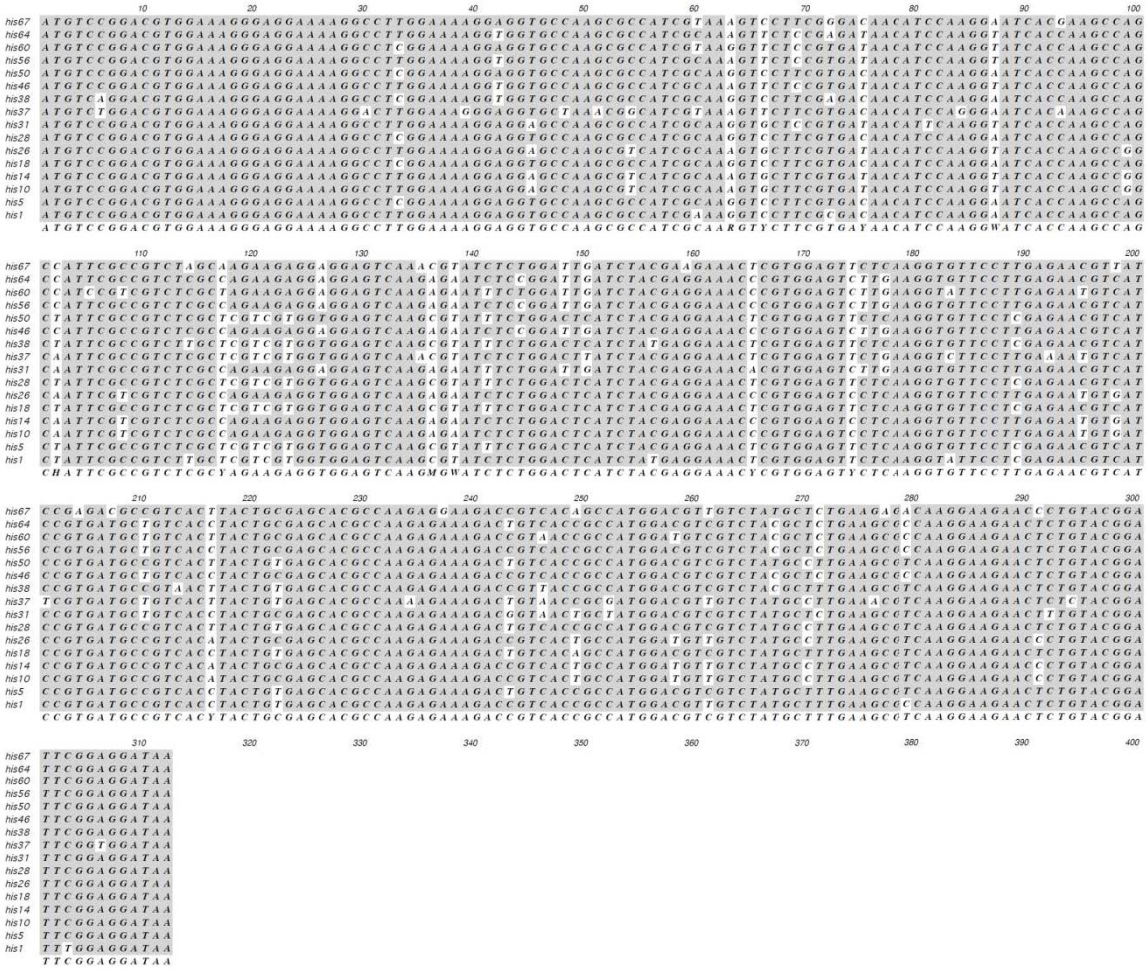


Figure 10. Multiple sequence alignment of histone H4 coding genes.

The histone H4 coding genes in *C. elegans* show great similarity. The sequence alignment was generated using MacVector software.

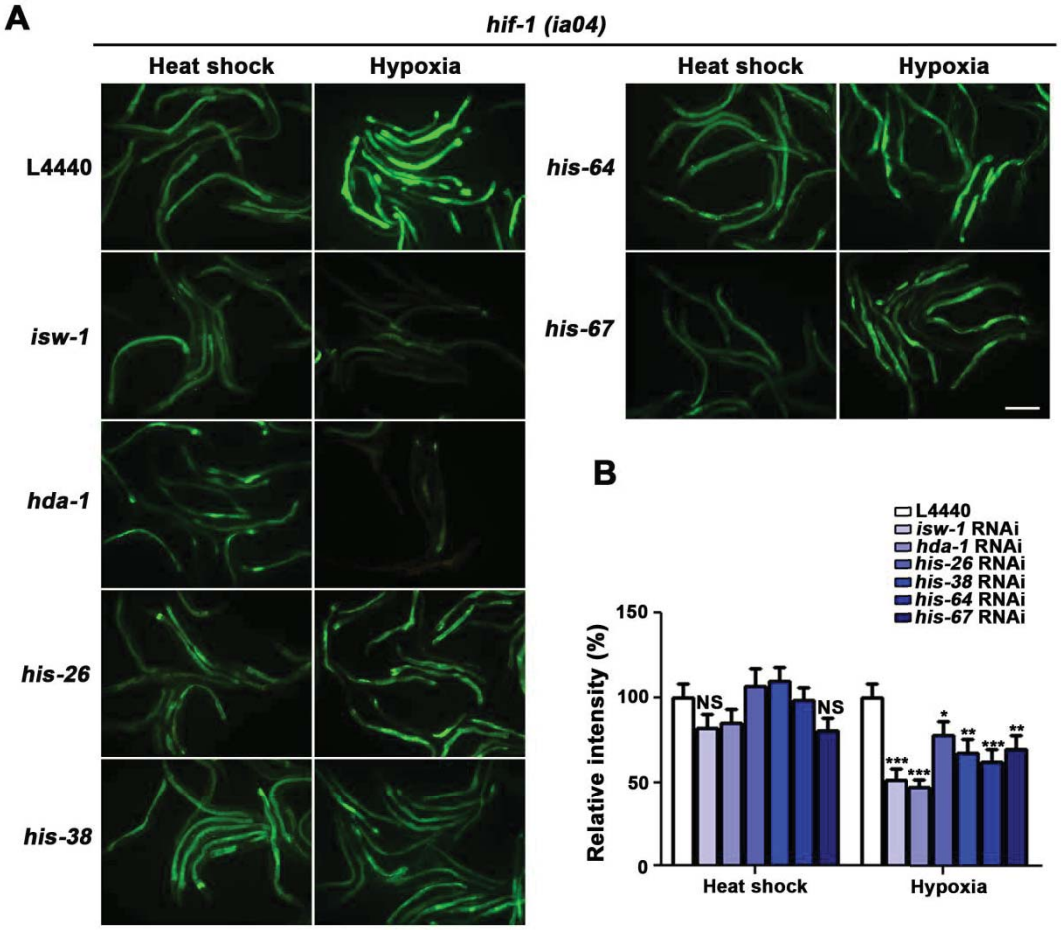


Figure 11. Regulation of *hsp-16.1* through histone modifiers and histone H4 does not require the function of *hif-1*.

(A) Reductions in *isw-1*, *hda-1*, *his-26*, *his-38*, *his-64* and *his-67* RNAi suppressed the hypoxia response of the *hsp-16.1 in hif-1 (ia04)* mutants. The scale bar represents 200 μm. **(B)** The quantification of the relative intensity of HSP-16.1::GFP fluorescence was carried out using ImageJ software. Statistical significance was determined using a one-way ANOVA and Dunnett′s multiple comparison with the L4440 control (* p ⟨0.05, ** p ⟨0.01, *** p ⟨0.001). NS, not significant. The error bars indicate the SEM.

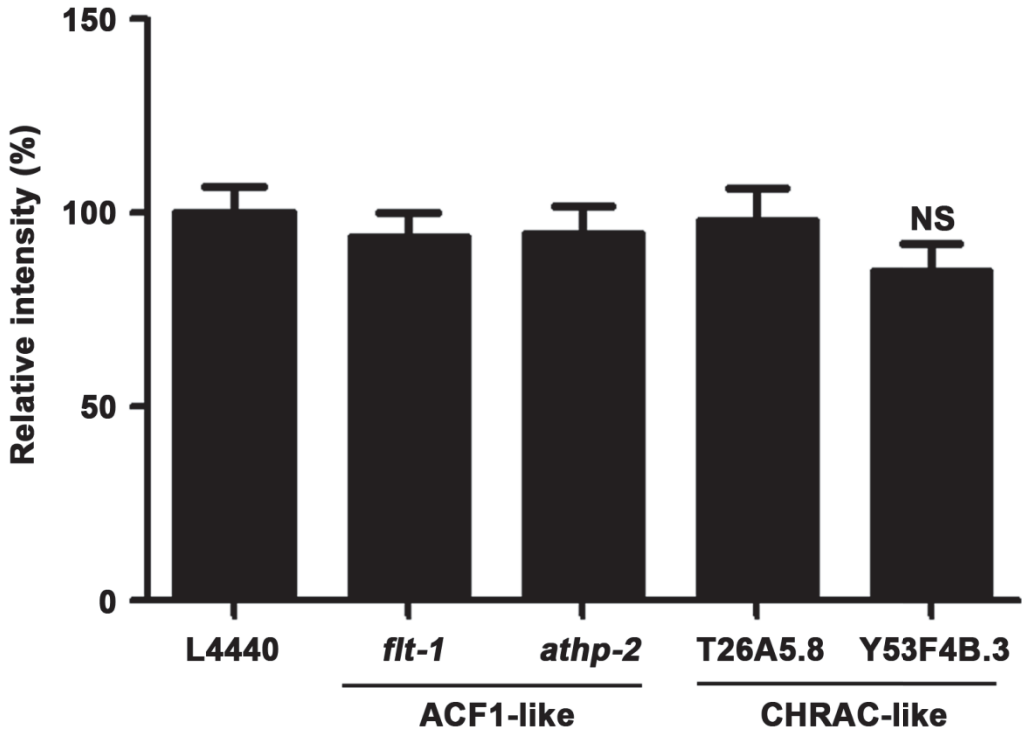


Figure 12. ACF and CHRAC genes are not required for the *hsp-16.1* hypoxia response. The inactivation of the orthologs of ACF- and CHRAC-like genes did not abolish the up-regulation of HSP-16.1::GFP by hypoxia. The relative fluorescence intensity of hypoxia-induced HSP-16.1::GFP was quantified using ImageJ software. Statistical significance was determined using a one-way ANOVA (Dunnett test). NS, not significant. The error bars indicate the SEM.

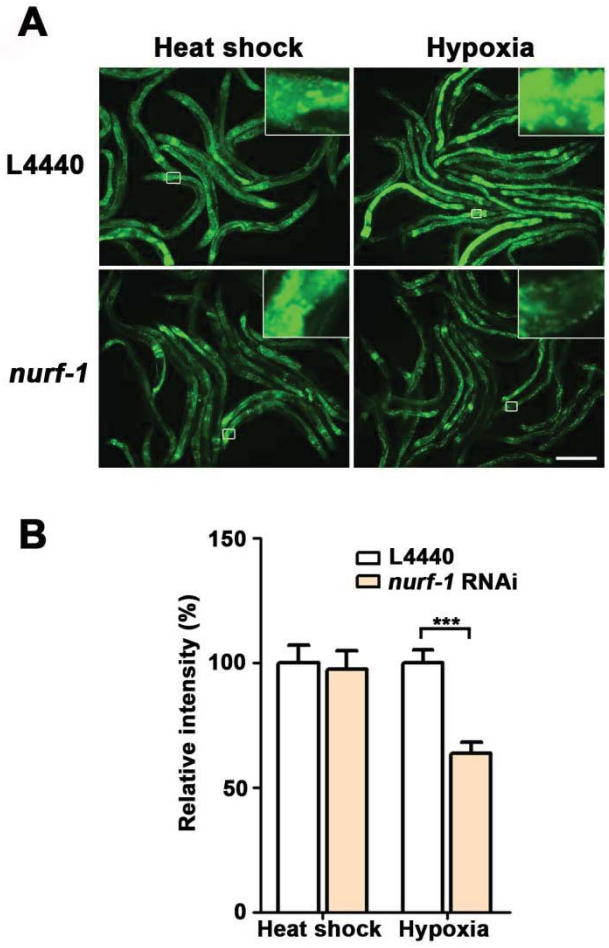


Figure 13. NURF-1 chromatin remodeling factor mediates the hypoxia response of the *hsp-16.1* gene.

(A) Decreased *nurf-1 function* (*Drosophila* NURF301) reduces the activation of *hsp-16.1* by hypoxia. The results for ACF and CHRAC RNAi are presented in **Fig. 12**. **(B)** The quantification of the relative intensity of HSP-16.1::GFP fluorescence was carried out using ImageJ software. Statistical significance was determined using an unpaired t-test (*** p 〈0.001). The error bars indicate the SEM. The scale bar represents 200 μm.

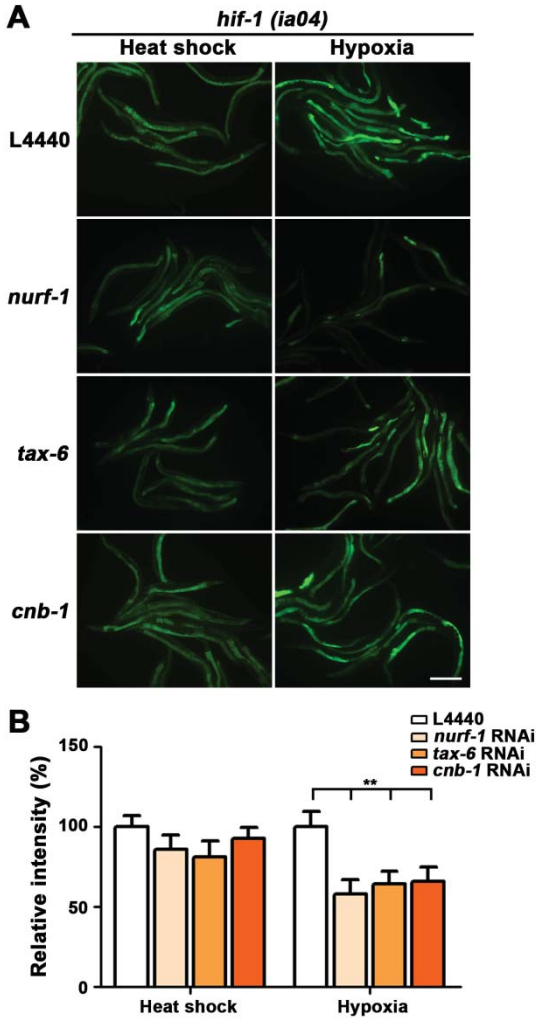


Figure 14. NURF-1 and calcineurin do not require HIF-1 for the hypoxic regulation of *hsp-16.1*. **(A)** Knockdown of *nurf-1* and calcineurin (catalytic subunit, *tax-6*; and regulatory subunit, *cnb-1*) in *hif-1(ia04)* mutant animals resulted in a consistent *hsp-16.1* hypoxia response in wild type animals. The scale bar represents 200 μm. **(B)** The relative fluorescence intensity of stress-induced HSP-16.1::GFP was quantified using ImageJ software. Statistical significance was determined using a one-way ANOVA (Dunnett test). ** $p < 0.01$. NS, not significant. The error bars indicate the SEM.

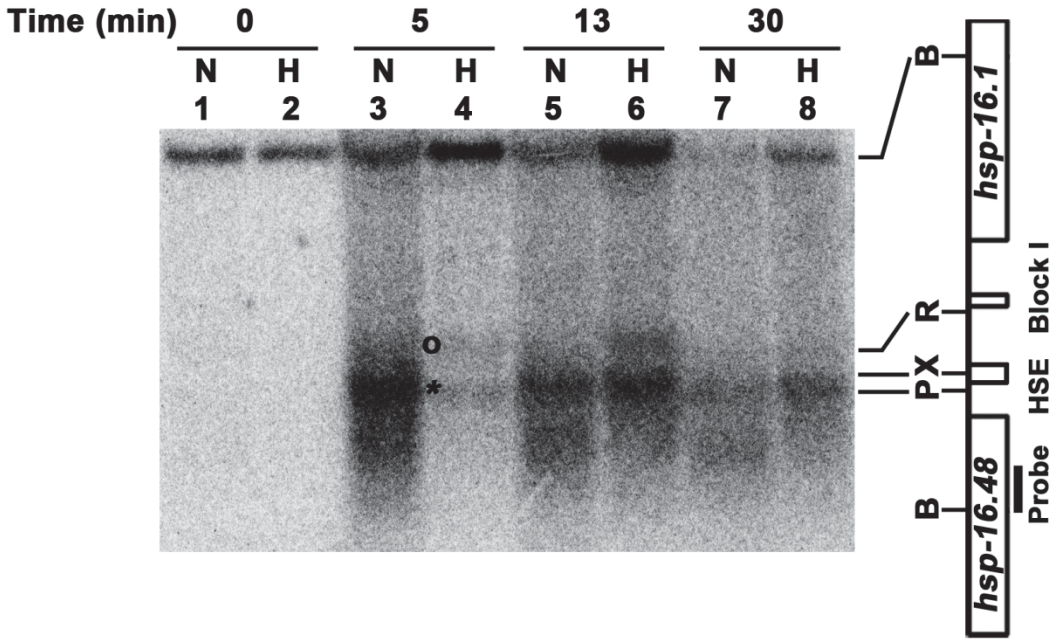


Figure 15. Hypoxia affects the chromatin arrangement on the promoter of *hsp-16.1*

MNase digestion patterns of the normoxic and hypoxic chromatins. The DNA of MNase-digested nuclei was digested by *Bgl*II and analyzed by Southern blot hybridization using a 200 nt-probe made from the region designated in the figure. Chromatin was incubated in presence of MNase for either 0 min (lanes 1 and 2), 5 min (lanes 3 and 4), 13 min (lane 5 and 6) or 30 min (lane 7 and 8). Asterisk indicates genomic position that is more sensitive to MNase cleavage in normoxia. Open circle indicates the band with increased sensitivity to MNase cleavage in hypoxia. N = normoxia, H = hypoxia. Restriction sites (B : *Bgl*II, P : *Pst*I, X : *Xba*I, R : *Rsa*I)

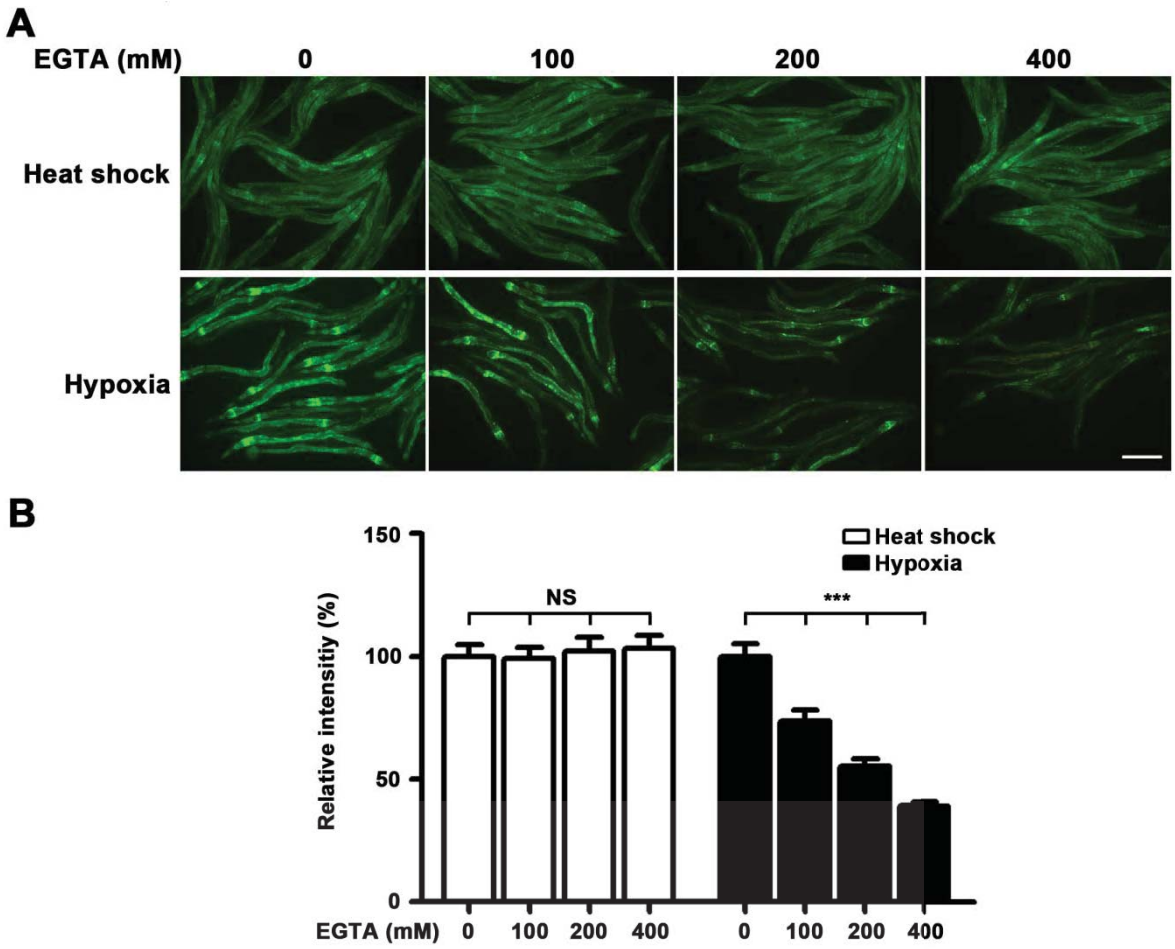


Figure 16. Manipulation of calcium balance affects hypoxia-induced *hsp-16.1* expression. (A) Treatment with EGTA, a calcium chelator at different concentrations (0, 100, 200 and 400 mM). EGTA-driven depletion of calcium decreased the induction of *hsp-16.1* under hypoxia but not under heat shock treatment. However, EGTA did not affect the heat shock-inducible expression of *hsp-16.1*. The scale bar represents 200 μm. **(B)** The quantification of the relative intensity of HSP-16.1::GFP. The triple asterisk indicates $p < 0.001$ significance level (Dunnett test).

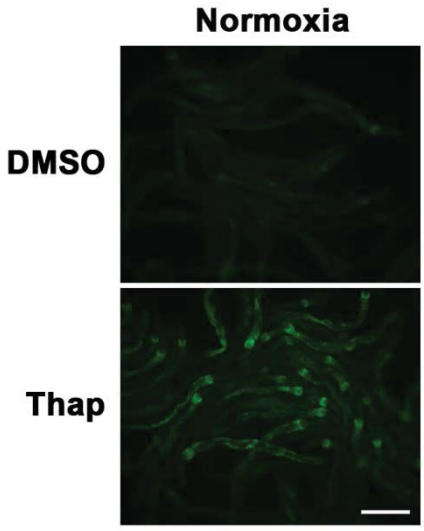


Figure 17. Thapsigargin treatment

Thapsigargin-induced Ca^{2+} release mimicked the effect of hypoxia to activate the expression of *hsp-16.1*. Same volume of solvent alone as a control (DMSO). Thap: Thapsigargin. The scale bar represents 200 μm.

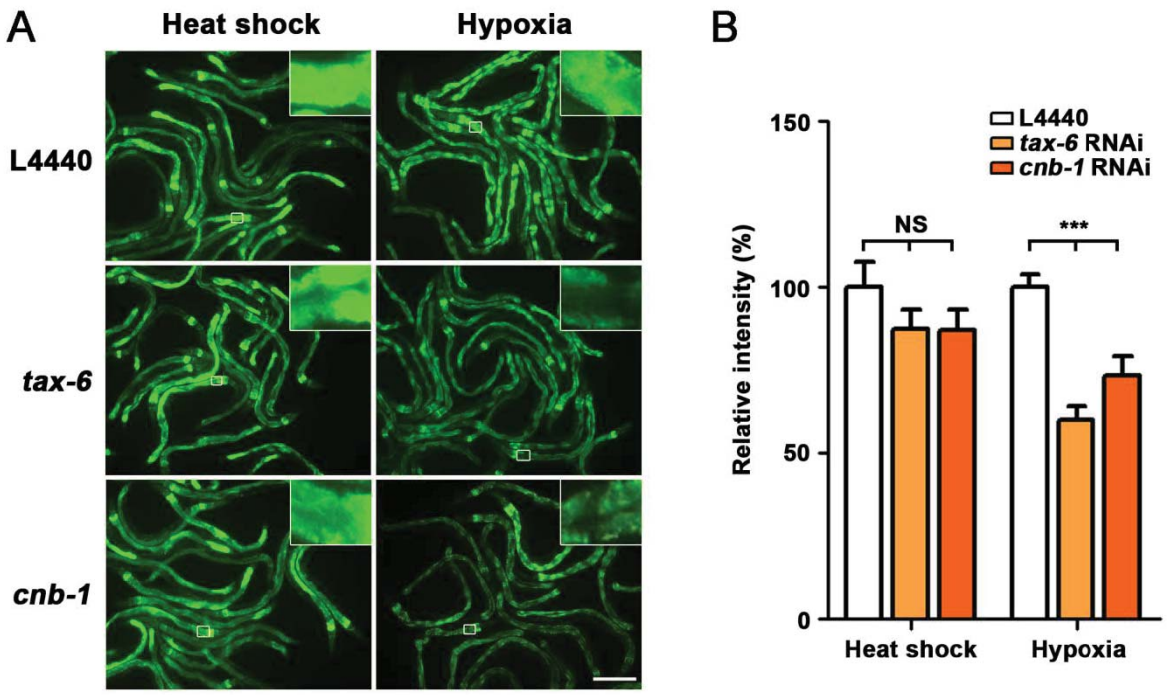


Figure 18. Cacineurin is required for the hypoxia response of *hsp-16.1*

(A) Inactivation of calcineurin decreased the activation of *hsp-16.1* under hypoxia. **(B)** The quantification of the relative intensity of HSP-16.1::GFP fluorescence was carried out using ImageJ software. The triple asterisk indicate values that differ from the L4440 hypoxia controls at the p 〈0.001 significance level (Dunnett test). NS, not significant. The error bars indicate the SEM. The scale bar represents 200 μm.

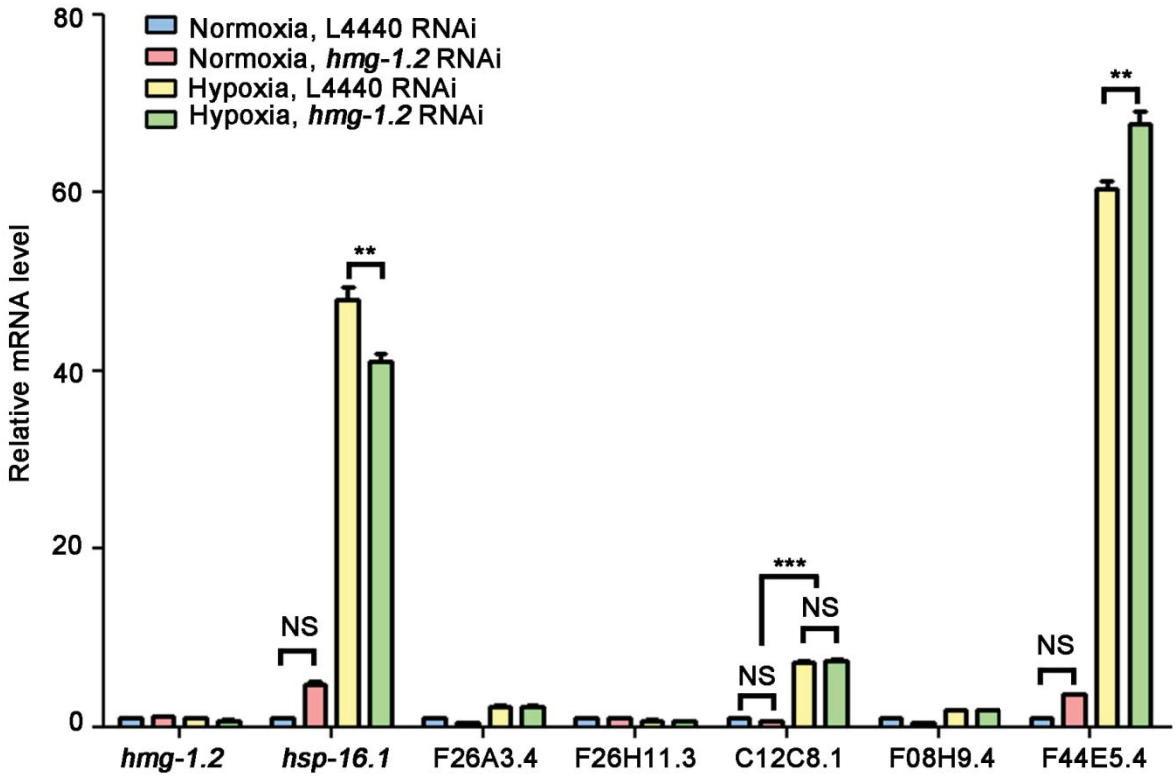


Figure 19. Relative mRNA levels of genes regulated by hypoxia in HIF-1-independent manner.

Relative mRNA levels were measured using Real-Time quantitative PCR analysis. Statistical significance was determined using a one-way ANOVA and Bonferroni′s multiple comparison (**p ⟨0.01, *** p ⟨0.001). NS, not significant. The error bars indicate the SEM.

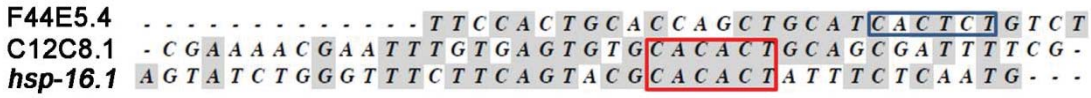


Figure 20. Comparison of block I regions in *hsp-16.1*, C12C8.1 and F44E5.4.

The block I sequence is conserved between *hsp-16.1* and C12C8.1. Red box : block I, blue box : block I-like region. The sequence alignment was generated using MacVector software.

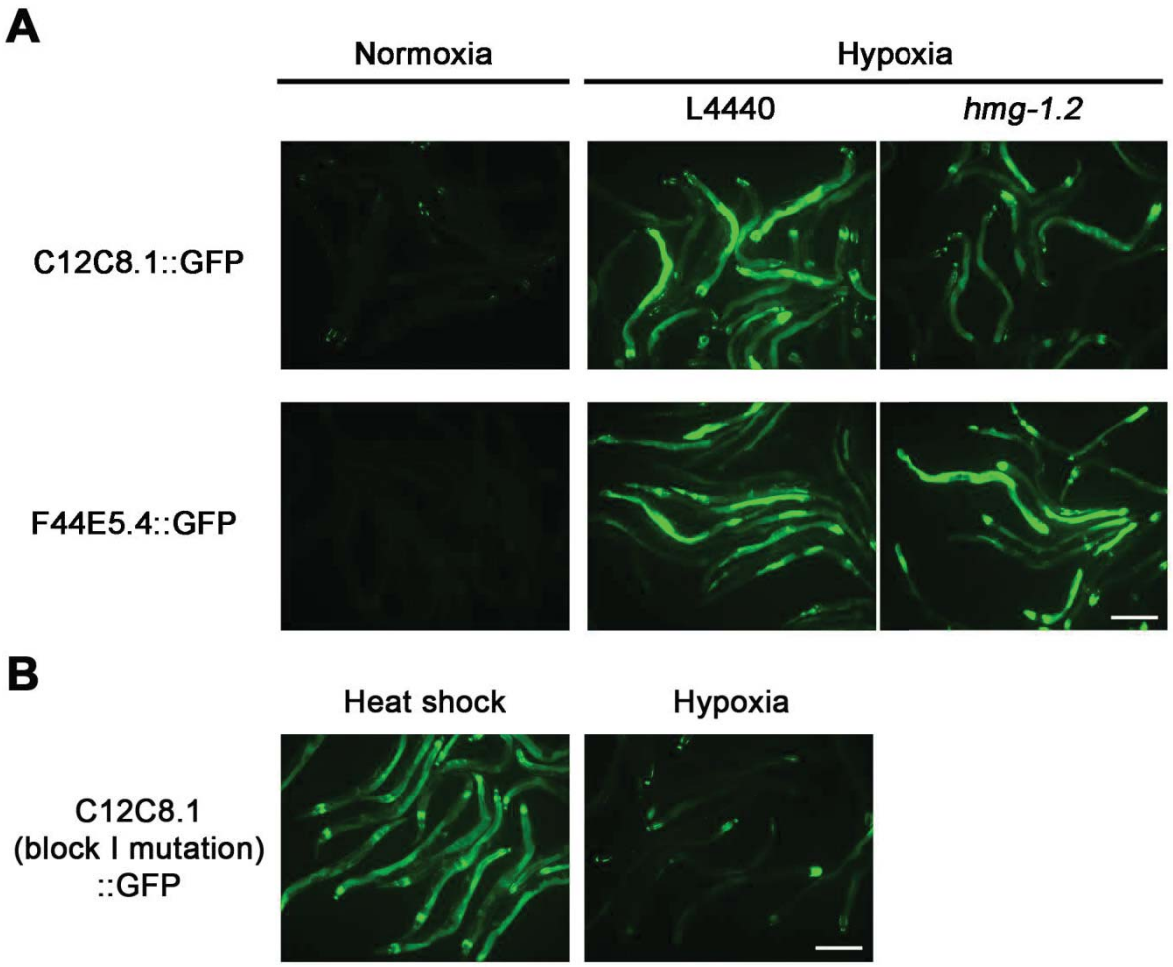


Figure 21. C12C8.1::GFP and F44E5.4::GFP are induced by hypoxia

(A) C12C8.1 is induced by hypoxia and the hypoxia response of C12C8.1::GFP is decreased by *hmg-1.2* RNAi, while F44E5.4::GFP is not altered by RNAi under hypoxia. **(B)** Animals carrying deletion of the conserved block I are responsive to heat shock, but not hypoxia. The scale bar represents 200 μm.

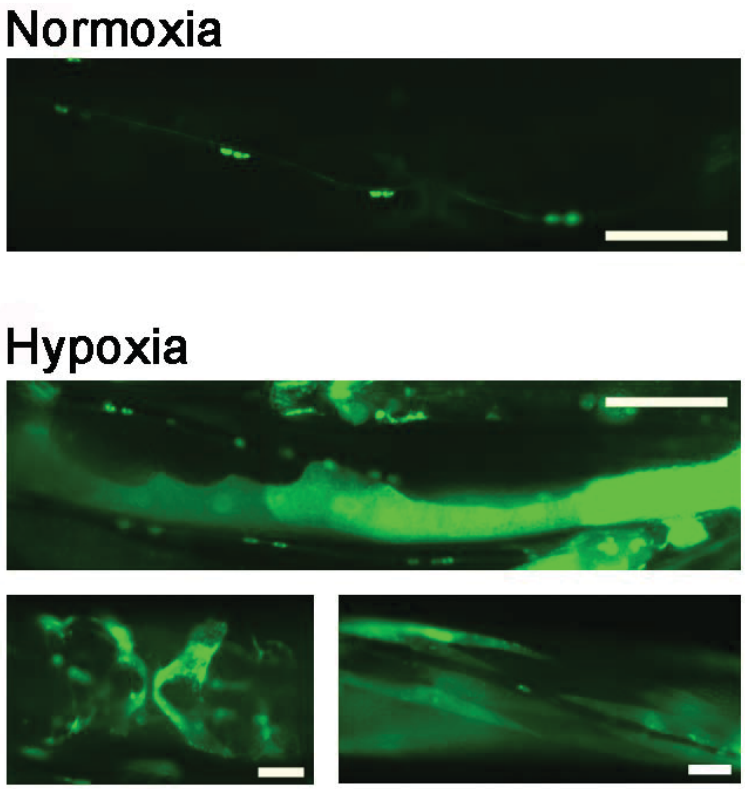


Figure 22. Expression patterns of C12C8.1::GFP

Under normal condition, C12C8.1 is expressed only in the ventral cord of *C. elegans*. C12C8.1::GFP is highly inducible by hypoxia. Intestinal cells, vulval muscle cells and body wall muscle cells are upregulated under hypoxia. The scale bars represent 10 μm.

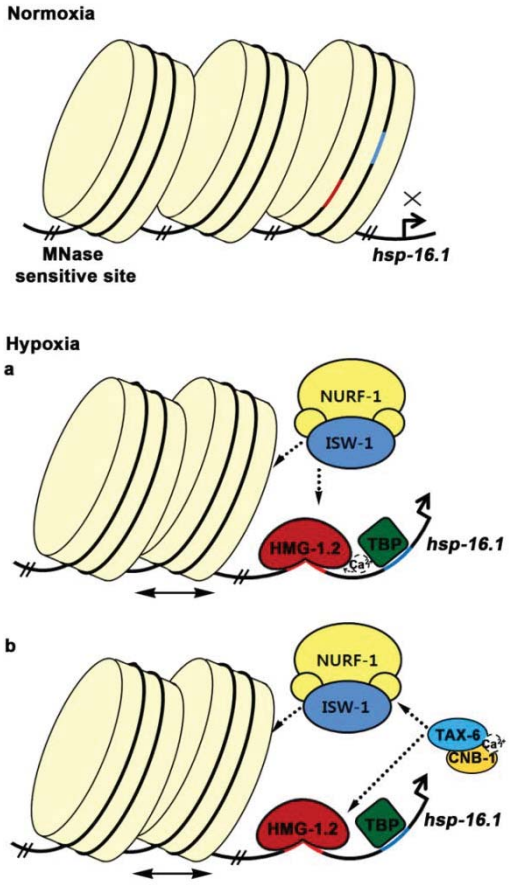


Figure 23. A model of HIF-1-independent hypoxia regulation of *hsp-16.1*.

Under normal oxygen conditions, the block I region (red line), surrounded by nucleosomes, may not activate the transcription of *hsp-16.1*. (a) Hypoxia induces the release of calcium from the endoplasmic reticulum and influences HMG-1.2 in a way that HMG-1.2 has a higher affinity for DNA. In concert with HMG-1.2, ISW-1 and the NURF-1 remodeling complex are involved in remodeling the positioning of the nucleosomes on block I. (b) Activated calcineurin (TAX-6 and CNB-1) might affect either ISW-1/NURF-1 or HMG-1.2, which results in nucleosome sliding at the promoter of *hsp-16.1*. A horizontal bi-directional arrow indicates a possible nucleosome sliding. Once HMG-1.2 binds to DNA, it can bend the DNA to allow efficient binding by several elements of the transcription machinery such as TATA-binding protein (TBP). TATA box is indicated as the blue line. Dashed lines indicate hypothetical regulation.

Table 1. A functional gene network with HMG-1.2

Gene	Experimental detection methods	Relative intensity[a]	References
hmg-4	Reconstituted Complex	103.41 (4.86)	(Lee et al., 2007a)
hmg-11	Genetic interaction, affinity Capture-MS	100.80 (6.86)	(Rual et al., 2005)
mab-5	Phenotypic Enhancement, Suppression	103.68 (7.22)	(Zhong and Sternberg, 2006)
ima-3	Two-hybrid	102.16 (6.81)	(Li et al., 2004)
his-1	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-5	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-10	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-14	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-18	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-26	Genetic interaction, affinity Capture-MS	69.42 (4.17)[c]	(Formosa et al., 2002)
his-28	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-37	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-38	Genetic interaction, affinity Capture-MS	65.94 (5.19)[c]	(Formosa et al., 2002)
his-46	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-50	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)

his-56	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-60	Genetic interaction, affinity Capture-MS	69.42 (4.17)[bc]	(Formosa et al., 2002)
his-64	Genetic interaction, affinity Capture-MS	61.54 (3.81)[c]	(Formosa et al., 2002)
his-67	Genetic interaction, affinity Capture-MS	64.91 (5.06)[c]	(Formosa et al., 2002)
psa-4	Genetic interaction	100.72 (6.59)	(Lee et al., 2007b)
htz-1	Affinity Capture-MS	119.73 (13.36)	(Lee et al., 2007b)
hpk-1	Biological process	102.54 (9.26)	(Rual et al., 2005)
isw-1	Affinity Capture-MS	79.93 (4.33)[c]	(Ewing et al., 2007)
rpa-1	Affinity Capture-MS, Biological process	93.21 (6.86)	(Zhong and Sternberg, 2006)
hda-1	Biological process	45.05 (4.95)[c]	(Zhong and Sternberg, 2006)
Y41E3.11	Two-hybrid	104.50 (4.09)	(Lee et al., 2008)

[a] Knockdown of the genes was performed using RNAi, and the expression levels of *hsp-16.1* were quantified using ImageJ software. The result of the L4440 control RNAi was 100 (SEM: 7.69). Statistical significance was determined using a one-way ANOVA (Dunnett test).

[b] Same RNAi effect with *his-26*

[c] $p < 0.001$

IV. Discussion

In this study, I sought to better establish the molecular mechanisms of HIF-1-independent hypoxia responses in *C. elegans*. I showed that chromatin remodeling factors including HMG-1.2 are involved in the *hif-1*-independent hypoxia response of *hsp-16.1* expression. In addition, I have shown that calcium is important for induction of *hsp-16.1* transcription in response to hypoxia. Taking all my data and previous studies together, I propose a mechanism for the HIF-1-independent hypoxia response (Fig. 23). As this regulatory mechanism may not act specifically on *hsp-16.1*, future study will be needed to investigate the extent to which this mechanism is involved in hypoxia-responsive regulation. Because chromatin remodeling factors are well conserved in evolution, it is conceivable that a similar mechanism of *hif-1*-independent hypoxia response may occur in mammals.

HMG-1.2 is a homolog of the human HMGB2 protein and exhibits approximately 55% identity within the HMG boxes (Jiang and Sternberg, 1999). HMGB proteins are non-histone chromatin-binding proteins that are generally thought to have limited sequence-specific DNA recognition ability, preferring to bind to bent DNA and four-way junction DNA (Bustin and Reeves, 1996). Nevertheless, evidence was found in *C. elegans* that HMG-1.2 might also have site-specific sequence recognition abilities, for example, in Wnt signaling in specific developmental processes (Jiang and Sternberg, 1999). The sequence-specificity of HMG-1.2 may allow it to regulate transcription of the *hsp-16.1* gene in oxygen-deprived conditions independently of HIF-1. It would be of a great interest to determine whether genes other than *hsp-16.1* and *-16.2* that contain the block I sequences at their promoter regions also require HMG-1.2 for their response to hypoxia. As this regulatory mechanism may not act specifically on *hsp-16.1*, I pursued to identify other hypoxia-inducible genes which are regulated dependently of HMG-1.2. Among the 47 genes whose expression was altered in *hif-1 (ia04)* mutants, only C12C8.1 and F44E5.4 were induced by hypoxia. Because the fold changes of other genes in microarray were subtle, it is probable that only C12C8.1 and F44E5.4 are potentially regulated in HIF-1 independent manner under hypoxia. Transcription of *hsp-16.1* and C12C8.1 is rapidly induced at early stage of ethanol response (Kwon et al., 2004). There is a block I sequence at

500 bp upstream from the C12C8.1 ORF (open reading frame) and the proximal regions of block I between C12C8.1 and *hsp-16.1* share high similarity. Further investigations are needed to determine whether the block I sequence at promoter of C12C8.1 also requires HMG-1.2 for its response to hypoxia. mRNA level of C12C8.1 was not reduced by *hmg-1.2* RNAi, while its protein level was decreased by reduction of *hmg-1.2*. Therefore, I cannot exclude the possibility that the hypoxia response of C12C8.1 could be regulated independently from *hmg-1.2*. On the other hand, although mRNA level of F44E5.4 was affected by *hmg-1.2* RNAi, F44E5.4 is unlikely to be a target of HMG-1.2 since there is no block I sequence on its promoter and its protein level was not affected by *hmg-1.2* RNAi under hypoxia.

A functional gene network enabled me to predict interplays between HMG-1.2 and chromatin remodeling factors. It is known that HMGB proteins bind to nucleosomes, thereby loosening the wrapped DNA and enhancing its accessibility to chromatin remodeling complexes (Bonaldi et al., 2002; Bustin and Reeves, 1996). HMGB proteins have also been suggested to accelerate the sliding activities of chromatin remodeling and to enhance the binding of chromatin remodeling factors to nucleosomal DNA (Bonaldi et al., 2002). In addition, recent genetic evidence has shown that HMG-1.2 is upregulated by hypoxia and that knockdown of *hmg-1.2*, *isw-1* and *hda-1* significantly increases sensitivity to hypoxia (Mabon et al., 2009). Although I could not detect a direct physical interaction between HMG-1.2 and ISW-1 using immunoprecipitation (data not shown), these studies support a relationship between HMG-1.2 and chromatin modifiers in hypoxic conditions. To further define epistasis between HMG-1.2 and chromatin remodeling factors, it would be interesting to perform double RNAi of *hmg-1.2* and other genes encoding interacting proteins. Moreover, the N-terminus of histone H4 is essential for stimulating ISWI ATPase activity and inducing nucleosome sliding (Clapier et al., 2001; Georgel et al., 1997). This suggests that histone H4 may act to modulate the activity of chromatin remodeler in the hypoxia response. It would be interesting to investigate whether the deacetylation at the N-terminal region of histone H4 proteins by HDA-1 is involved in this

modulation.

My data suggest that ISW-1 may act with NURF chromatin remodeling factors to modulate the hypoxia response of *hsp-16.1* independently of HIF-1 and that NURF-mediated unwrapping of the block I region by nucleosome movement may contribute to the activation of *hsp-16* under hypoxia conditions. Additionally, *nurf-1* was upregulated by hypoxia in *hif-1*-deficient animals (Shen et al., 2005). This implies that NURF-1 is regulated under hypoxia independently of HIF-1. Unfortunately, because the null mutants of *hmg-1.2*, *isw-1* and *nurf-1* show severe defects in both development and fertility, I cannot determine whether the nucleosome remodeling is abolished in these null mutants in vivo. However, ISWI and NURF are known to promote nucleosome sliding at promoters, which leads to the disruption of regularly ordered arrays and thereby activates gene transcription (Hamiche et al., 1999; Mizuguchi et al., 1997). Given this knowledge, I propose that the chromatin remodeling complex of ISW-1 and NURF-1 catalyzes the nucleosome sliding at the *hsp-16.1* promoter to facilitate transcriptional activation and that transcriptional regulation via nucleosome and chromatin modifications is important for the regulation of the hypoxia response. As knockdown of *pyp-1* and *rba-1* resulted in embryonic lethality, I could not test whether these genes were involved in the hypoxia response.

My finding that the hypoxia response of *hsp-16.1* is calcium-dependent is consistent with the notion that calcium release is a landmark of early hypoxia response. Endoplasmic reticulum (ER) is a major Ca^{2+} store and it is known that Ca^{2+} release is mediated by 1,4,5-triphosphate (IP_3) receptor Ca^{2+} channel in the ER (Berridge et al., 2003). To investigate a cause of calcium increase, I knockdowned *itr-1*, *crt-1*, *unc-68*, and *sca-1*, *C. elegans* homolog of the IP_3 receptor, calreticulin, a calcium binding chaperone of the ER, ryanodine receptor and SERCA, respectively. I found that none of them affected the upregulation of *hsp-16.1* by hypoxia (data not shown). There are several possible explanations for how *hsp-16.1* expression is regulated by calcium signaling under hypoxic conditions.

First, an increase in the intracellular calcium levels increases the affinity of HMG-1.2 for DNA, which is necessary to induce the hypoxia response of *hsp-16.1*. Another possibility is that calcineurin activated by a high cytosolic calcium level may dephosphorylate unidentified substrates like ISW-1, which in turn positively modulates the HIF-1-independent hypoxia response of *hsp-16.1*. To further define whether calcineurin regulates ISW-1, it needs to be accompanied that knockdown of calcineurin alters the expression or localization of either HMG-1.2 or ISW-1. It would be of interest to pursue the issue of calcium action mechanism in hypoxia response.

HIF-1α is overexpressed in common human cancers, in cells in the center of a solid tumor, which experience hypoxia (Zhong et al., 1999). Although HIF-1α has been considered to be a major target for tumor therapy, HIF-1 is not the only regulator of the hypoxia response in cancer cells (Mizukami et al., 2006). My findings propose a novel, alternative mechanism that regulates gene induction in hypoxia in a HIF-1-independent manner. Because *C. elegans* cellular adaptations to hypoxia are homologous to those found in mammals, HMG-1.2 and the ISW-1 chromatin factor may be therapeutic targets, in addition to HIF-1, and could block hypoxia-induced responses in a combinatorial manner in cancer cells.

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APPENDIX I

Primers in this study

hsp-16.1-1	CCAAGCTTGAGCATTCTTGAAGTTTAGAG
hsp-16.1-2	CGCGTCGACTTCAGAAGTTTTTTGTTCAACG
blockI affinity-1	CGTGGTTTCTTCAGTACGCACACTATTTCTCAATGTTC
Tsub affinity-1	CTGGGTTTCTTCAGTACGTTTTTTATTTCTCAATGTTC
hsp-16.1 ChIP-1	AGGTGCAAAGAGACGCAGAT
hsp-16.1 ChIP-2	CTAGAACATTCGAGCTGCTT
hmg-1.2-1	AAACTGCAGGTGCTCTTTCACTTCCTCTG
hmg-1.2-2	CGCGTCGACGTCATAATGGTCCATGCCAG
hda-1-1	ATCTAGACTCTGCATTGAATGATGACTC
hda-1-2	AAACTGCAGCTCTGTCTTCTGACGCTTTTC
nurf-1-1	ATCTAGACAGAATCCACATCATCATCTC
nurf-1-2	AAACTGCAGAGAAACGGTAGCTCATGAAG
hsp-16.48 probe-1	AGATCTTCTGGTTTGAAATGAG
hsp-16.48 probe-2	GGAGAGGTAAGAAAATAATC
isw-1-1	AGGATCCAGTTTATCAGTTGCCTGCTTG
isw-1-2	AACCGGTTTAGGAGTAGCTTTGACTTTC
cnb-1-1	ATCTAGAAAGTCGACTTCAGAGGTGAC
cnb-1-2	AAACTGCAGATGTCGTAGATGCGGAATGC
H20J04.2_1	ATCTAGATGATGATTTCGACTTGACAAGC
H20J04.2_2	AAACTGCAGTCTTCATAGCCTCGGAAATTAC
f26a3.4_1 qpcr-1	CTGCGTTGAGCAAAGATGAA
f26a3.4_2 qpcr-2	CAGATGGAAGCAGAACGTGA
f26h11.2a_1 qpcr-1	AATGACGGAGACGAACAACC
f26h11.2a_2 qpcr-1	GGATCACGTTGTGTTTGACG
c12c8.1_1 qpcr-1	GCTGATCTTTTCCGCAAGAC

c12c8.1_2 qpcr-1	CCAAAGGCTACTGCTTCGTC
f08h9.4_1 qpcr-1	GGCTTATGCCAATTTCTGGA
f08h9.4_2 qpcr-1	TCCACGTCATGTTCTCCTTG
f44e5.5_1 qpcr-1	CTTGTGAACGTGCCAAGAGA
f44e5.5_2 qpcr-1	AATTTTCGGAACACGAGTGG
hmg-1.2_1 qpcr-1	GAATTCTGGTTACAGCGCAAA
hmg-1.2_2 qpcr-1	ATGGAGATGTCTTGCCTCGT
hsp16.1-1 qpcr-1	CAATGTCTCGCAGTTCAAGC
hsp16.1-2 qpcr-1	GCAACTGCACCAACATCAAC
f44e5.4_1	AAGCATGCGTAGTAGTAGAACAGTAGAAC
f44e5.4_2	AAGCATGCTATTCCACTGCACCAGCTGC
c12c8.1_1	AAAGTCGACTATTTGACACTCTGCCATATC
c12c8.1_2	AGGATCCATCAACTTCCTCTACAGTAGG
c12c8.1_gaattc1	CGAATTTGTGAGTGTGGAATTCGCAGCGATTTTCGAGGCGC
c12c8.1_gaattc2	GCGCCTCGAAAATCGCTGCGAATTCCACACTCACAAATTCG

국문초록

이지현

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정상적인 발달의 과정에서 또는 질병이 발생하면, 세포와 개체는 정상보다 낮은 산소농도인 저산소 상태에 놓이게 된다. 저산소에 의해 발생하는 손상과 괴사를 막기 위해서 세포 수준에서 많은 유전자의 발현이 변화한다. 이들 유전자는 HIF-1에 의해 조절된다고 알려져 있다. HIF-1에 의존하지 않는 저산소 반응 유전자의 조절이 보고된 바가 있지만, 분자수준의 메커니즘에 대해서는 거의 알려진 바가 거의 없다. 최근에는 예쁜꼬마 선충을 이용한 저산소 스트레스 반응 메커니즘이 활발히 연구되고 있고, 이러한 연구는 포유류에게도 응용되고 있다. 이 논문에서 예쁜 꼬마 선충의 HMG-1.2와 크로마틴 리모델링 요소들이 HIF-1 독립적인 방법으로 저산소에서의 유전자의 발현을 조절하는데 중요하다는 점을 밝혔다.

HIF-1은 저산소 반응 유전자의 발현을 조절하는 필수 전사인자이지만, 최근에는 HIF-1에 독립적으로 저산소 상태에서 반응하는 유전자로서 small HSP (heat shock protein) 유전자를 포함하는 새로운 유전자들이 발견되었다. HIF-1 독립적인 저산소 반

응에 필요한 cis-acting (block I) 요소가 *hsp-16.1*의 promoter에 존재하고, 예쁜 꼬마 선충의 용해물에는 이 block I에 특이적으로 결합하는 요소가 있음이 알려졌다. 따라서 *hsp-16.1*의 block I에 결합하는 조절 인자의 발굴하고자 친화성 크로마토그래피와 질량 분석기를 이용하였고, 그 결과, 이 조절 인자는 HMG-1.2임을 확인하였다. 또한, 염색질 면역 침강법을 수행하여 HMG-1.2는 생체 내에서 저산소 반응 특이적으로 *hsp-16.1*의 promoter에 결합하는 것을 알 수 있었다.

HMG-1.2는 조인자 (cofactor)이기 때문에, HMG-1.2와 상호 작용하는 전사활성인자 또는 복합체의 구성요소를 규명하고자 '유전체 네트워크'를 도입하였다. 이는, 두 유전자가 물리적 혹은 유전적으로 상호작용한다면, 그들 유전자는 같은 조절 경로에 존재한다는 가설을 바탕으로 한다. 유전체 네트워크는 다른 동물 모델 (효모, 초파리) 와 표유류에서 밝혀진 mRNA 발현 양상, 물리적·유전적 상호작용, 같은 논문에 거론된 유전자들의 예쁜 꼬마 선충의 상동유전자들과 같은 정보를 통합하여 특정 유전자와의 상호작용 가능성을 제시해 줄 수 있다. 이를 통해, HMG-1.2와 직·간접적으로 상호작용하는 후보 유전자를 확보하였다. 유전자 간섭 실험을 수행하여 크로마틴 리모델링에 관여하는 ISW-1/NURF-1 복합체가 저산소 반응에 필요함을 규명하였다. 이를 바탕으로 저산소에 의해 크로마틴의 리모델링이 유발되는지 알아보고자 하였다. 뉴클레오솜에 감싸여 있지 않는 DNA만 특이적으로 자르는 효소인 마이크로코칼핵산내부가수분해효소 (micrococcal endonuclease)를 처리한 후, Southern blot을 수행하여 저산소 반응이 *hsp-16.1* 유전자의 promoter에 존재하는 뉴클레오솜의 배열을 변화시키는 것을 밝혔다. 특히 HMG-1.2가 결합하는 block I 부분이 저산소 반응에 의해 뉴클레오솜으로부터 분리되는 것이 *hsp-16.1* 유전자의 발현에 중요한 것으로 보여진다. 마지막으로, 저산소 반응 중에 하나로써 세포내 단백질의 항상성을 조절하는 세포소기관인 ER (Endoplasmic

reticulum)로부터 칼슘 이온이 방출되면, 칼슘을 매개로 하는 신호전달에 의해 하위 유전자가 조절 받게 된다. 이와 관련하여 칼슘 이온과, *tax-6*와 *cnb-1* 복합체가 HIF-1 독립적인 저산소 반응에 필요한 것을 알 수 있었다.

이러한 결과들을 통해 HIF-1 독립적인 저산소 스트레스 반응은 HMG-1.2를 매개로 하며, ISW-1/NURF-1 크로마틴 리모델링 복합체와 칼슘 신호전달의 영향으로 조절됨을 알 수 있었다. 이러한 결과들을 바탕으로 저산소 스트레스 반응은 저산소 상태에서의 세포 기능 제어과정을 정확히 이해하는데 도움이 될 것으로 기대하고, 나아가 세포 기능을 임의로 제어 가능하다면 저산소에 의한 심장 손상이나 암세포의 치료에 도움이 될 가능성이 있음을 생각해 볼 수 있다.

주요어 : 예쁜 꼬마 선충, HIF-1 비의존적 저산소 반응, *hsp-16.1*, HMG-1.2, 크로마틴 리모델링 요소, 칼슘 이온

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