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Differential Hypoxia Response of the hsp-16 Genes in the Nematode

Mingi Hong[P1], Jae Young Kwon[1], Jaegal Shim[1], Junho Lee[C1]

NRL, Department of Biology, Yonsei University, Seoul 120-749

Eukaryotic cells respond to various stresses by the induction of new sets of proteins including a group of heat shock proteins (HSPs). Among these, small heat-shock proteins (smHSPs) form a diverse family of proteins that are produced in all organisms in sizes ranging from 12 kDa to 43 kDa. It is known that diverse types of stress increase the expression level of small HSPs, such as salt stress, oxidative stress, and nutrient depletion as well as heat shock. The *C. elegans* genome contains 16 genes encoding 14 distinct smHSPs. Among them, HSP-16.1/HSP-16.48 and HSP-16.2/HSP-16.41 are the major HSP-16 proteins. These four HSP-16s are very similar in gene structure and amino acid sequence. As in other organisms, the *C. elegans* hsp-16 genes have been reported to be induced by many types of stress such as reactive oxygen species (ROS), beta amyloid peptide, heavy metals, and oxidative stress. Hypoxia is another type of stress for cells and organisms. Since hypoxia has been studied mostly in hypoxic tissues and cells, the genes identified to respond to hypoxia have been limited to the genes expressed in those tissues. In addition to genes in specific tissues, it is conceivable that general stress response proteins such as heat shock proteins respond to hypoxia to overcome the stress. Indeed, it has been reported that hypoxia induces HSP70 and HSP90 family proteins in various systems. However, it is not known whether small HSPs are also induced by hypoxia. We aimed at characterizing the response of the hsp-16 genes to hypoxia using the nematode as a model system. In a microarray experiment in which expression profiles of all the predicted genes in *C. elegans* were compared before and after ethanol treatment, we found that HSP-16 proteins were strongly induced by ethanol. Since heat shock proteins are thought to play roles in various stress conditions, we hypothesized that these HSP-16 proteins are general stress response proteins. *We found that two of the hsp-16 genes in C. elegans* responded to hypoxia, while the other two genes, which share the promoter regions with their counterparts, did not. As a comparative genomic approach, we examined the hsp-16 genes in two closely related nematode species, *C. elegans* and *Caenorhabditis briggsae. C.briggsae* has been proven to be a good model to study functional conservation of the genes in the nematodes. We identified 10 hsp-16 genes in the nematode *C. briggsae* from the genome database. Phylogenic analysis of the amino acid sequences and the promoter sequences of the 10 cb-hsp-16 genes with those of the *C. elegans* hsp-16 genes established that the HSP-16 family could be classified to two classes. The comparison of the promoter sequences revealed a new conserved sequence block that was required for the orientation-dependent hypoxia response, but not for other stress responses such as heat or ethanol. As reported previously for the *C. elegans* hsp-16 genes, the heat shock response elements (HSEs) are also conserved in the *C. briggsae hsp-16 genes*. There were two more conserved regions, which we named ESRE (ethanol and stress response element), that are also located symmetrically. The ESRE sequences were partially identified by GuhaThakurta et al. as a novel heat shock element, and by ourselves as a regulatory element required for ethanol and stress response. Because the HSE and ESRE elements, which are symmetrically located, should act in both directions, these elements can not be the specific regulatory element required for the orientation-dependent hypoxia response, although they may be necessary in part. We therefore looked for additional conserved elements located proximally to the distal ESRE. In addition to the conserved HSE and ESRE elements, we found two more conserved blocks of sequences that were located between the proximal ESRE and HSE elements in only one direction of the promoter sequences. We found that deletion of the block I, but not the block II, resulted in the loss of hypoxia response without affecting the heat shock or ethanol responses of hsp-16.1, indicating that the block I sequence is required for hypoxia response. We supposed that a factor bound to the orientation dependent sequences might drive the transcriptional machinery preferentially towards one direction, but not the other. In order to examine the existence of the factor that binds the block I sequence, we performed electrophoresis mobility shift assay with the *C. elegans* nuclear extract. There was a specific binding factor to the 19 bp sequence containing block I in vitro. We propose a working model for the orientation-dependent promoter usage between two genes sharing the promoter region. We also discuss a possible application of the hypoxia-inducible promoter for conditional gene expression.