Molecular Genetic Study on Transcriptional Mediators Using Caenorhabditis elegans transgenics

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Mediators are the transcriptional cofactors originally isolated from the yeast. We are interested in the functions of mediators in metazoans because biological significance of mediators in metazoan development or transcription system has not been examined yet. In the hope of elucidating the functions of mediators in development, we started examination of functions of mediators in the nematode Caenorhabditis elegans. We cloned a med-6 homolog in C. elegans by database search and RT-PCR. We then investigated the expression patterns of Ce-MED6 by antibody staining. Ce-MED6 is expressed in the peripheral region of the nuclei of all cells at all developmental stages, where actively transcribed portions of the genome are supposedly localized. For the functional analysis of Ce-MED6, we performed RNA interference experiments. The phenotypes of the progeny from the wild-type animals injected with double-stranded RNA were embryonic lethality, arrested larvae, and sterility, indicating that Ce-MED6 is required for many aspects of development including embryogenesis and germ cell development. It is crucial to test whether the transcription of any tissue- or stagespecific genes is affected by Ce-MED6 malfunction in vivo. To this end, we looked into the effect of RNAi on the transcription levels of ceh-13 and nhr-2. The expression of these genes are lineage- or stage-specific and were down-regulated by Ce-Med6 RNAi. In contrast, ribosomal protein and sur-5 that are constitutively expressed didn't show remarkable differences from normal expression levels. Let-425 may be identical to the Ce-Med6, because a nonsense mutation is found in the coding region of Ce-Med6 from the let-425 mutant. We found that Ce-MED6 colocalizes with RNA polymerase II in a big complex in vivo by biochemical methods. We are also trying to identify and characterize more mediators in C. elegans, for example, Ce-Med7, Ce-Med10, and Ce-Srb7, and will also present the functional analysis of these genes.