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Heat Inducible Expression of the *CDC70* Gene under the Control of Heat Shock Element in *Saccharomyces cerevisiae*

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In order to express the *CDC70* gene of *Saccharomyces cerevisiae* by heat shock, we have designed heat inducibe hybrid promoters using the *Drosophila melanogaster* heat shock elements (HSEs). A 220-bp-long upstream fragment of the *D. melanogaster hsp70* gene comprised of four HSEs was placed upstream of the putative proximal TATA box of the *CDC70* gene. Hybrid promoters containing different fusion joints were tested for their ability to drive the *CDC70* gene expression by heat shock. The results showed that the HSEs of *D. melanogaster* conferred the heat-induced *CDC70* gene expression, but the heat inducibility was much lower than that in *D. melanogaster*.

Key words: heat shock element, *hsp70* gene, *CDC70* gene, heat inducible expression

Various kinds of inducible promoters have been used, not only to study functions of genes, but also to produce scientifically or commercially valuable proteins in both prokaryotic and eukaryotic organisms. Among them, a group of heat shock promoters have been a powerful candidate for the inducible promoters, especially since it can make genes over-expressed, and an inducer for the promoter can be easily added and removed by the laboratory scale (14). Heat shock promoters of *hsp* genes of *D. melanogaster* which encode heat shock proteins are well studied (17, 19, 20, 23, 26). Several heat shock elements (HSEs) responsible for the heat inducibility are present in the promoter regions of the *hsp* genes (19). Transcription factors, referred to heat shock transcription factors (HSTFs) have been purified from yeast and *D. melanogaster*. The HSTFs isolated from yeast and *D. melanogaster* are similar in molecular weight and in binding affinity to HSEs, suggesting that the mechanism of heat shock response is evolutionary conserved (24). Supporting this hypothesis, the *hsp70* gene of *D. melanogaster* can be expressed by heat shock, when tranfected into mouse cells, monkey cells, and sea urchin embryo, or injected into *Xenopus* oocytes (19).

Several sequence elements are known to be necessary for eukaryotic promoters to transcribe genes efficiently. These sequence elements can be grouped largely into two groups: general promoter elements and specific promoter elements. General promoter elements are comprised of TATA sequence element, CCAAT sequence, and GC motif. The TATA sequence element is located at 25~30 bp upstream of transcription initiation site. Several kinds of trans-acting transcription factors binding to these sequence elements have been identified in many eukaryotic organisms (18, 22). Specific promoter elements include HSE, TGACT motif, and in yeast promoters, upstream activating sequence (UAS) (2, 4, 7, 11), and in mammalian promoters, glucocorticoid response element (GRE) and metal response element (MRE) (16, 21). Although the HSE of *D. melanogaster hsp* gene, of which the sequence is C_GAA_TTC_G, is 1.4 helical-turn upstream of the TATA box, the distance between the HSE and the TATA box does not affect heat inducibility (12, 13). Moreover, the TATA sequence and the transcription initiation site are dispensible, when the HSEs are placed in the control region as multiple copies (13). However, both of them as well as the HSE are required for heat inducibility, when one copy of HSE exists (8, 25). These results suggest that the HSEs in multiple copies are similar to enhancers (1).

In this study, we investigated whether the HSE of

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D. melanogaster hsp70 gene could be used to place the yeast *CDC70* gene under the heat shock control.

Materials and Methods

Bacterial strains and plasmids

E. coli HB101 and *S. cerevisiae* 381Gα6 (*Matα ade6 his4 lys2 trp1 tyr1*) were used (9). Plasmids pGEM3 and pMC1871 were obtained from Promega Co. (Madison, Wisconsin, USA) and Phamacia Biotech Co. (Uppsala, Sweden), respectively. The yeast *CDC70* gene was in a recombinant plasmid pBR322-H(1.9) (9). The transformation vector into yeast was YRp7 (9). The HIC-UP cassette (Fig. 1) used has a sequence from -256 to -38 of *D. melanogaster hsp70* gene, where four HSEs are located, but does not contain a TATA sequence, requiring a TATA sequence from the foreign gene which is to be expressed in the downstream of the cassette (13).

DNA technology

All DNA manipulations were carried out as described by Maniatis *et al.* (15), unless noted otherwise.

Deletion by *Bal*31

A putative transcription regulatory sequence of the yeast *CDC70* gene was removed by *Bal*31 nuclease digestion. A *CDC70-lacZ* construct (Fig. 2) was linearized with *Kpn*I, followed by *Bal*31 treatment at 30℃ for different incubation periods. Several deletion derivatives of the construct were obtained. The derivative products were determined by *Pvu*II digestion and analyzed on a 5% polyacrylamide gel within the error margin of 5 bp.

Transformation of yeast

Yeast was transformed as described by Hinnen *et al.* (6).

Gene expression induction by heat shock

Yeast cells were cultured at 23℃ to $A_{600}=0.5$, followed by heat shock for 1 hr at 37℃. After heat shock, cells were incubated for another 1 hr at 23℃ to have the cells recover from heat shock. As a control, cells were cultured at 23℃ without heat shock.

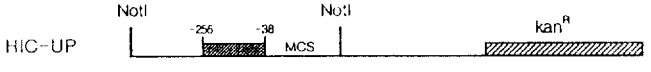

Fig. 1. Diagram of the HIC-UP cassette. The hatched box indicates the HSE elements of the *Drosophila hsp70*. Multicloning site (MCS) contains restriction enzyme recognition sequences for *Sal*I, *Cla*I, *Hin*dIII, *Eco*RV, and *Eco*RI.

Assay of β-galactosidase

The β-galactosidase activity of the yeast cells was determined as described by the method of Guarente and Ptashne (5).

Results

Construction of hybrid genes

The *E. coli lacZ* gene encoding β-galactosidase has been used as a reporter gene to investigate regulation systems of various genes. We constructed a *CDC70-lacZ* hybrid gene, resulting in, possibly, a production of CDC 70-LacZ hybrid protein having β-galactosidase activity under the control of the *CDC70* gene promoter (Fig. 2). In this construct, the *E. coli lacZ* open reading frame (ORF) was inserted, in frame, into the *Hin*dIII site of the *CDC70* ORF sequence. The 1.9 kb DNA fragment containing the *CDC70* gene obtained from pBR322-H(1.9) by digestion with *Eco*RI was blunt-ended with mungbean nuclease, and then ligated with pGEM3-H linearized with *Sma*I, to generate pGEM3-H(1.9). The pGEM3-H was made by digestion of pGEM3 with *Hin*dIII followed by treatment of mungbean nuclease to make the DNA blunt-ended and then religated. In order to insert the

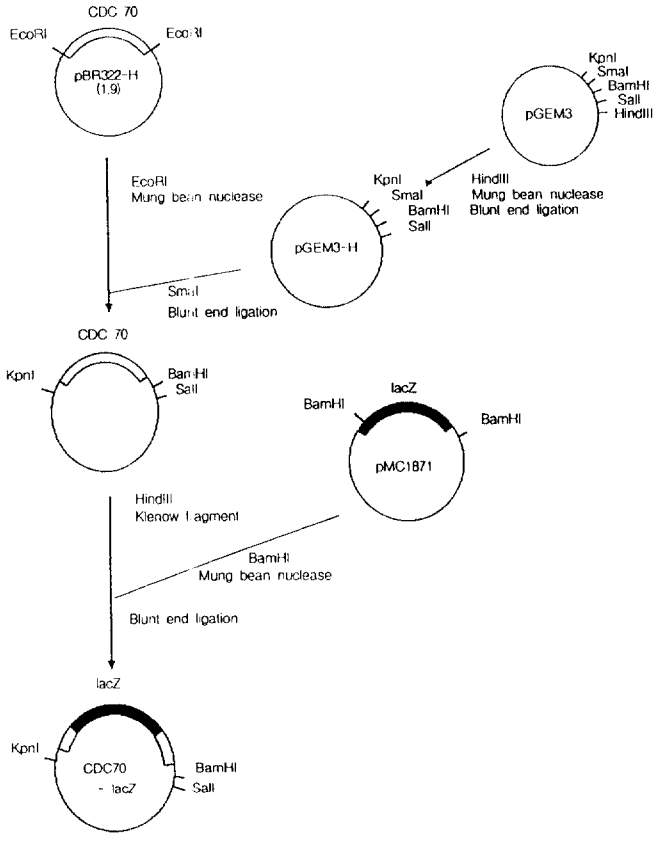

Fig. 2. Construction of *CDC70-lacZ* fusion. The open and filled boxes indicate the *CDC70* gene and the *lacZ* gene, respectively.

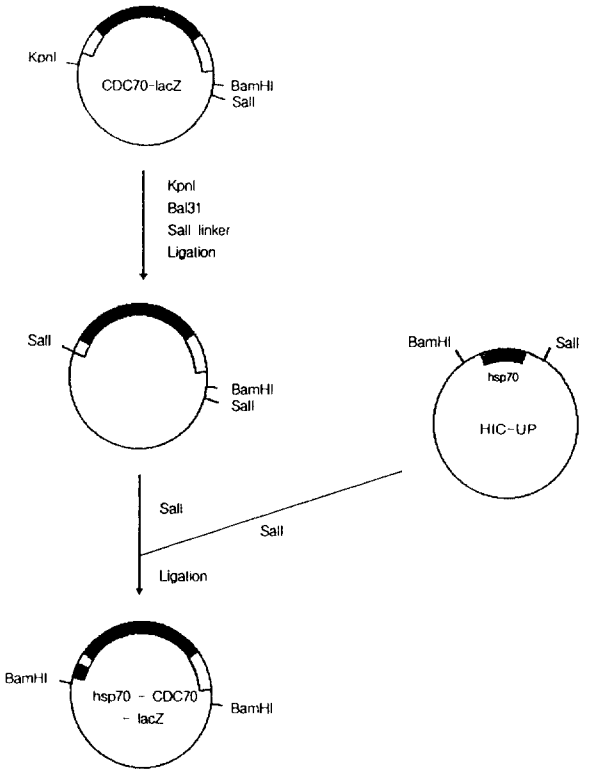

Fig. 3. Construction of *hsp70-CDC70-lacZ* fusion. The hatched box indicates the HSE of the *Drosophila hsp70*. The other description of the figure is the same as that of Fig. 2.

lacZ gene into the *CDC70* gene, a 3.1 kb DNA fragment in pMC1871 containing the *lacZ* gene was obtained by digesting pMC1871 with *Bam*HI, blunt-ended with mungbean nuclease, and ligated with a pGEM3-H(1.9) linearized with *Hin*dIII and blunt-ended by filling the recessive 3′ end with Klenow enzyme, to generate the *CDC 70-lacZ* fusion gene. From the fused construct, the control region of the *CDC70* gene upstream of the *CDC70* TATA box, was deleted with *Bal*31 enzyme after digesting the construct with *Kpn*I in which the recognition site is 5′ upstream of the *CDC70* gene. After ligating the octamer *Sal*I linker to the deleted constructs, the *Sal*I fragments from the deleted constructs were inserted into the HIC-UP cassette which contained the HSE of the *hsp70* gene (Fig. 3). Thus, two fusion constructs, *hsp 70-CDC70-lacZ1* and *hsp70-CDC70-lacZ2*, were obtained. The deletion end points of the two constructs were −45 and −10, respectively, from the putative transcription initiation site of the *CDC70* gene. Finally, the *Bam*HI fragment of each of the two constructs was transferred to the *Bam*HI site in an yeast vector YRp7, followed by transformation of the recombinant plasmids into yeast.

Expression of the fusion constructs by heat shock

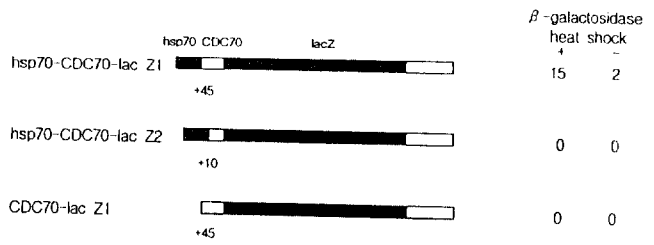

Fig. 4. The β-galactosidase activity expressed from *hsp70-CDC70-lacZ* constructs. The unit of β-galactosidase is expressed as described by Guarente and Ptashne (5).

The β-galactosidase activity of yeast cells transformed by *hsp70-CDC70-lacZ* constructs were measured. After cells were heat-shocked, the recovery period was provided for an enzyme assay, because the translation of mRNA for the non heat-shocked cells was very poor at high temperature. As shown in Fig. 4, *hsp70-CDC70-lacZ1*, containing the control region from −45 to −1 of the *CDC70* gene, showed an 8-fold increase in the β-galactosidase activity after heat shock. In contrast, the *CDC70-lacZ* construct, which was similar to the *hsp70-CDC70-lacZ1* but contained no HSE, did not show detectable β-galactosidase activity. This result indicates that the upstream sequences from −200 to −46 of the *CDC 70* gene would be required for the promoter activity because the *CDC70* gene containing the upstream sequence extending to −200 has shown to be capable of complementing the *cdc70* mutation in yeast (11). In this sequence, a distal TATA box appears at −174 position. The *hsp70-CDC70-lacZ2* construct, having only the sequence from −10 to −1 of the *CDC70* gene, also did not show the β-galactosidase activity independently of heat shock, possibly because the proximal TATA box at −27 position was absent in the construct.

Discussion

In this study, the HSE of *D. melanogaster hsp70* gene was used to express the yeast *CDC70* gene by heat shock. The *hsp70-CDC70-lacZ1* construct having the control region of the *CDC70* gene from −45 to −1 showed an 8-fold increase in the β-galactosidase activity after heat shock. Although this increase is very low compared to *D. melanogaster* which has more than 150-fold increase (14), the *D. melanogaster* HSE seems to be, at least, partially functional in yeast. Consistent to this result was that the HSE is evolutionary conserved and that the *D. melanogaster* HSTF and the yeast HSTF show similarity in binding affinity to HSEs (14, 24). The low level induction by heat shock was explained by the following possibilities. First, in the *hsp70-CDC70-lacZ1* construct,

regulatory region including a distal TATA box at position −174 was removed, which possibly resulted in the low level induction. Alternatively, the induction level in yeast may not be as high as in *D. melanogaster*, because basal expression was so high.

A construct of *CDC70-lacZ1*, which lacks a control region of the *CDC70* gene from −200 to −46 and any HSE, showed no detectable β-galactosidase activity, indicating that the control region is important in the promoter activity. When the control region was deleted from −200 to −11, the HSE did not express heat-induced β-galactosidase activity. This result suggests that the proximal TATA sequence of the *CDC70* gene acts as a TATA element in a construction of the *hsp70-CDC 70-lacZ1*. However, this does not mean that the actual transcription initiation site in this construct was same as that in the *CDC70* gene.

In conclusion, this study showed that the *D. melanogaster* HSE can induce a gene in the yeast system, if the gene is properly placed downstream of the HSE. If various parameter including the number of the HSEs in the control region, position of the HSE insertion in the promoter sequence, presence of a TATA sequence and the distance between the HSE and a TATA sequence are optimized, the induction level by heat shock is expected to be high enough to overexpress a specific gene in yeast system.

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