Two or more copies of Drosophila heat shock consensus sequence serve to activate transcription in yeast

Randolph Wei, Hilary Wilkinson, Karl Pfeifer, Carrie Schneider, Richard Young and Leonard Guarente

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

ABSTRACT
     A synthetic oligonucleotide bearing the Drosophila heat
shock consensus sequence confers heat inducibility on a
CYC1-lacZ gene in Saccharomyces cerevisiae. This sequence
CTGGAATTTTCTAGA was inserted in place of the upstream
activation sites of the CYC1 promoter adjacent to CYC1 TATA
boxes. These constructs were transformed into yeast and
found to be heat-inducible when two or more inserts were
present. The level of inducibility seemed to increase with
the number of inserted sequences: however, the orientations
of these sequences relative to each other did not have much
effect.

INTRODUCTION
     Heat shock proteins (hsp) have been found in almost
every organism investigated from bacteria to humans (1).
Hsp's are transiently expressed when cells are subjected to
high temperature or other stressful conditions. The
increase in hsp levels in heat shocked Drosophila is due to
both an increase in transcription of hsp genes and
preferential translation of hsp RNA (2, 3). These proteins
are presumed to protect the cells against deleterious
effects of high temperature or other forms of stress.
     The sequence of at least one hsp, hsp70, has been
highly conserved throughout evolution. Genes homologous to
hsp70 are found in multiple copies in yeast (4) and in the
form of the dnaK gene in E. coli (5, 6).
     The hsp's have been studied most extensively in
Drosophila. The promoter of the Drosophila hsp70 gene has
proved to be heat inducible in mouse tissue culture cells,
monkey COS cells, and Xenopus oocytes (7). The regulation
of heat shock genes has thus been highly conserved. The

conservation appears to be due to the presence of a regulatory sequence of consensus CTNGAANNTTCNAG, found in promoter regions of hsp70 and other Drosophila hsp genes (8). In hsp70, this sequence is found between -49 and -62 and between -72 and -85 (from the start of transcription) in two copies. This consensus, the heat shock element, confers heat shock inducibility when introduced on a synthetic oligomer upstream of the TATA box of the herpes thymidine kinase gene in monkey COS cells and Xenopus oocytes (9).

In yeast, several genes related by DNA homology to the hsp70 gene appear to be functionally distinct, i.e., some are required for growth at high temperatures while others are required for growth at low temperatures (10). Furthermore, those hsp70-like genes required for yeast growth at high temperatures are thermally induced.

RESULTS AND DISCUSSION

We investigated whether the DNA conservation in the heat shock induction response extends to yeast. Our approach was to examine the ability of synthetic oligonucleotides containing the heat shock consensus sequence to replace the upstream activation site of the CYC1 promoter region, which normally supports heme induced transcription of that gene (11, 12). This approach has been previously applied to identify UAS's for yeast genes such as GAL10 (13) and HIS4 (14).

To proceed, kinased oligonucleotides containing the heat shock element were ligated in place of the UAS sites on plasmid pLG-178, which contains the TATA boxes of CYC1 adjacent to a CYC1-lacZ fusion (Figure 1). The presence of inserts in each construct was initially determined by restriction digests and the sequences of several insert bearing plasmids were determined using M13 DNA sequencing analysis. Plasmids containing single, double, triple and quadruple inserts of the oligonucleotide were transfected into yeast strain BWG1-7A and β-galactodase levels were measured at the physiological temperature of 30 degrees and at the heat shock temperature of 39 degrees (Figure 1).

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TCGACTGGAATTTTCTAGA ────────▶
    GACCTTAAAAGATCTAGCT                                              30°C   39°C
                              XhoI   T    T     T   CYCI  lacZ
                              ┬──────────────────┬────●────       < I    < I
                             -178  -106  -52  -22
                       ──▶      ────────────────────┬────●────     < I    < I
                       ◀──      ────────────────────┬────●────     < I    < I
                    ──▶ ──▶      ───────────────────┬────●────     < I     35
                 ──▶ ──▶ ──▶      ──────────────────┬────●────     < I     75
                 ──▶ ◀── ──▶      ──────────────────┬────●────      3      75
              ◀── ──▶ ──▶ ──▶      ─────────────────┬────●────      3     105
                  UAS I  UAS 2
                  ┬──────┬──┬       ─────────────────┬────●────    203    166
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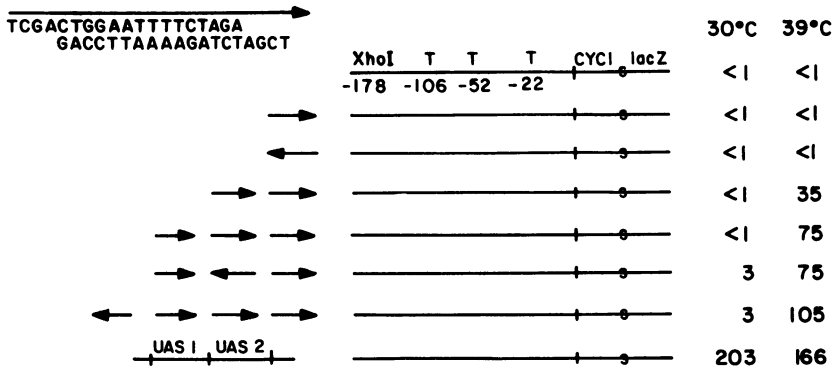
Figure 1 Construction of Yeast Heat Shock UASs.   The
oligonucleotide TCGACTGGAATTTTCTAGA
                    GACCTTAAAAGATCTAGCT
indicated by the arrows, was ligated into the plasmid pLGΔ-
178 (11) which had been cleaved by XhoI at -178. pLGΔ-178
contains the three CYC1 TATA boxes (T) (21) adjacent to a
CYC1-lacZ fused gene, as indicated, but is missing the CYC1
UAS. Inserts were screened, initially by restriction
analysis. A StuI-BamHI fragment spanning the insert was
isolated from several clones, inserted into the M13
derivative, MP18, and the DNA sequence of inserts determined
by dideoxy sequencing. Plasmids bearing the inserts shown,
along with pLGΔ-312 (bearing the CYC1 UASs) were transformed
into yeast BWG1-7a (11) and beta-galactosidase assayed as
described in the text. Units are according to Miller (22).

We found that plasmids with a single insert in either
orientation were inactive under heat shock conditions, as
was the pLG-178 parent plasmid, but plasmids bearing
multiple inserts gave heat shock inducible activity at
levels roughly proportional to the number of inserts
present. The orientation of the inserts did not affect the
overall level of heat induction; however, it did affect the
non-heat shocked level of activity. In those plasmid
bearing inserts that lay in both orientations the basal
level of activity was 10-20 fold greater than the basal
level produced by plasmids containing the same number of
inserts all in the same direction.
     Further experiments were also performed to determine
the kinetics of induction. The plasmid pLGA1-5 bearing
three inserts oriented in the same direction was employed in
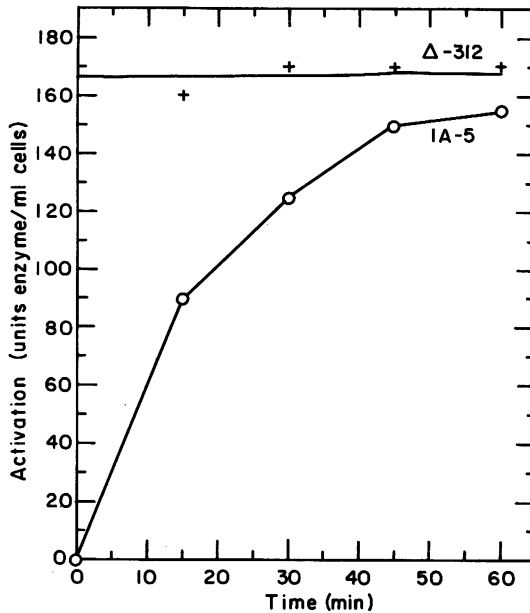this experiment. It was found that the heat shock response

Figure 2 Kinetics of the Heat Shock Response. Strain BWG1-7a (12) bearing either pLGΔ-312 or pLG1A-5 carrying three inserts oriented in the same direction, were grown at 30°C. At time 0, cultures were shifted to 39°C in a shaking water bath, and aliquots were sampled for beta-galactosidase activity at the indicated times.

occurs rapidly within the first half hour and that no significant increase in activity occurred after 45 minutes (Figure 2).

These results imply that the heat induction response is conserved from higher eukaryotes down to yeast. Two or more inserts of the heat shock element are required for this response. This finding is consistent with in vitro studies indicating binding of a Drosophila factor to two adjacent sites (15). It is also consistent with in vivo analysis in which deleted constructs were introduced back into flies (16, 17, 18, 19, 20).

The fact that more than one insert is required for activity and that the level of induction increases with the number of inserts suggests one of two possibilities. Either there is cooperative binding occurring between proteins that

bind to adjacent inserts, as has been found in the case of Drosophila described above (15), or activation requires the binding of two or more proteins to the UAS. In contrast, one insert appears to suffice for induction in monkey COS cells and <u>Xenopus</u> oocytes (7). This finding could either be due to differences in regulatory mechanisms in the organisms or to the differences in the locations of the insert with respect to the TATA box. In Pelham's experiments, the consensus sequence was positioned 12 or 13 base pairs from the TATA box, while in pLG-178, inserts were placed about 80 nucleotides upstream of the nearest TATA element. It would be of interest to compare the range over which the heat shock element is functional in yeast to that of endogenous UAS's (roughly 500 base pairs).

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