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Cloning of a cDNA encoding a novel heat-shock protein from *Dictyostelium discoideum* *

(Nucleotide sequence; small heat-shock protein; developmental expression)

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SUMMARY

We have cloned, from *Dictyostelium discoideum*, a cDNA encoding a new heat-shock (HS) protein (Hsp) with a predicted molecular mass of 31 447 Da. Outside of its low molecular mass, this Hsp does not share any similarity with the small Hsp currently identified or with α -crystallins. Northern blot analysis indicates that this HS-inducible gene is also developmentally regulated. < p >

All organisms ranging from bacteria to man respond to heat by inducing the synthesis of a group of proteins called the heat-shock proteins (Hsp). Nearly all species synthesize Hsp belonging to three different gene families, with sizes of 80–90, 68–76 and 15–40 kDa. The proteins belonging to the 80–90 and 68–76-kDa families are highly conserved; the predicted aa sequence of human Hsp70 is 73 and 50% identical to that of *Drosophila* and the *Escherichia coli* homologs (Bardwell and Craig, 1984; Mues et al., 1986). The low- M_r Hsp, however, are much less conserved in sequence and size. The proteins iden-

tified in eukaryotes appear to be related to each other and to the vertebrate α -crystallins (Lindquist and Craig, 1988) in that they have similar hydrophathy profiles and small regions of aa identity. The most conserved domain is the sequence (Xaa)₂-Gly-Leu-(Xaa)₃-Pro found near the C terminus at a similar position in the hydrophathy profiles of each protein (Lindquist and Craig, 1988). They also share the property of forming highly polymeric 15–20 S structures, often called HS granules.

While screening a λ gt11 cDNA library enriched for transcripts expressed preferentially in growing cells or the earliest hours of development, we isolated a cDNA clone encoding for a novel Hsp (Fig. 1) of 281 aa and 31 447 Da. The start ATG is preceded by six A's, agreeing with the observation that in *Dd* genes at least one A (and as many as 14 A's) precedes the Met start codon (Kimmel and Firtel, 1983). Furthermore, the codon usage of the ORF is in agreement with *Dd* codon pattern (Kimmel and Firtel, 1983). The ORF is terminated by a stop codon (TAA) at nt 873. The cDNA seems to be incomplete in its 3' UTR, since no poly(A) tail is present. However a putative polyadenylation site can be observed at the very end of the cDNA.

Northern blot analysis shows that an approx. 1-kb

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* On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note.

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Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, complementary DNA; *Dd*, *Dictyostelium discoideum*; HS, heat shock; Hsp, HS protein(s); kb, kilobase(s) or 1000 bp; nt, nucleotide(s); ORF, open reading frame; S, sedimentation constant; UTR, untranslated region(s); Xaa, any aa.

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TATTCCATATATACATATACATAAAAAATGATGCAATTTTTTGGTACTATCGTAACT 59
      M M Q F F G T I V T 10
AAAGAAGAACCAGTAAATCTTGAATTAGATGAAGGTGATATTTCCATTTAACTAAAGCT 119
K E E P V N L E L D E G D I F H L T K A 30
ATTATTCATCCAAAATCTCAAGGTAAGGTAAGTTTATTTAACTGCAGTTATTAGTTTA 179
I I H P K S Q G K G K V Y L T A V I S L 50
ATGGAAGAAGATGAAATGGAAGAAGATGATGTTGATGATGAAGAAGAAATCCCAAGAGAA 239
M E E D E M E E D D V D D E E E S P R E 70
GATATAGTTGAAAATCCCAATGGTATTTTAGAAGCTGGTAAAATGATCAAAATCGATCTC 299
D I V E I P I G I L E A G K I D Q I D L 90
AATTACATTATAATTTGGACAAATCGTTAGATTTGAACTCCAAGCTGAAAATGGTGCT 359
N L H Y N F G Q I V R F E L Q A E N G A 110
GGTATGTTGGTGGCTCTTCTGGTTCAGTTATGACCATGGAACAAGGTGGTGTGATGAT 419
G Y V V A L S G S V I T M E Q G G C D D 130
GAAGATTGTGATGATGAACATGCATCAATCATGAAGATGATGAAGAAATCGATAGTGAT 479
E D C D D E H C I N H E D D E E I D S D 150
GAAGAGTTCGGTGACTCTGATCAAGATGAAGAAGATTCCGATGATGAAGAATCCACAAA 539
E E F G D S D Q D E E D S D D E E I P Q 170
TTAATTGCCACCGCCACTAAAAAGGTAAGTAAATCACAGAATCTCAGAAGTCCCAGAATCA 599
L I A P A T K K G K I T E I S E V P E S 190
AAGAAGAAAAAATCCAGAACCAAGAAAGTTCCAGAACCAAGAAAGAAACAAGTTAAA 659
K K E K T P E F K K V P E P K K E Q V K 210
CAACCAACCCCAACCAACCAAAAAGAGTGTGCTCAACCAACCAAGAAAAGCCCAACAT 719
Q P T Q P Q Q K K A A A Q Q P E K A N N 230
AAACCGCCGCTGCTTCCACGCTAAACCAAAAACAATCAATCTAAAAATGCACCAAAA 779
K P A A A S P A K P Q N N Q S K N A P K 240
CAACCACAACAACAACAATCAACGACCAAAAATAATAACAACAAGACCACAAAAC 839
Q P Q Q Q Q Q S P A K N N N N K R P Q N 260
CAAAACGAAAAACAACAAAAGAAAACAAAAAATAAATAGTCAATAAA 887
Q N E N N K K K Q K N * 281

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Fig. 1. Nucleotide sequence of cDNA 4-1 from *Dd* and deduced aa sequence. The nt sequence was determined on both strands using the dideoxy chain-termination method (Sanger et al., 1977). The putative polyadenylation site is underlined. The sequences reported in this paper have been deposited in the GenBank™ EMBL Data Bank with accession No. L39778.

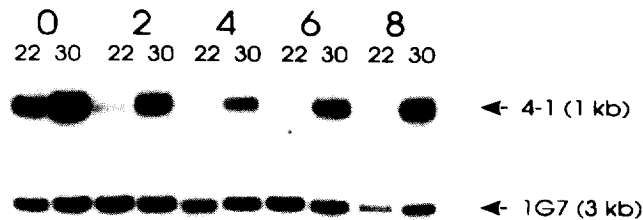


Fig. 2. 4-1 mRNA levels during development and after HS. Autoradiograms of the blot are shown. **Methods:** Cells were starved in 20 mM phosphate buffer to initiate development. After 0, 2, 4, 6 and 8 h of starvation at 21°C, some cells were transferred to 30°C for 30 min. Total RNA was isolated and 10 µg/lane analyzed by Northern blots (2.2 M HCOH 1% agarose) and probed with ³²P-labeled 4-1 cDNA. For a loading control, the Northern blot was also hybridized to ³²P-labeled IG7 cDNA, which represents a constitutively expressed gene (Early and Williams, 1988). Both probes were labeled by the random priming method of Feinberg and Vogelstein (1983).

mRNA, transcribed from this gene, is present at high levels in growing cells but decreases dramatically during the early hours of development (Fig. 2). If cells at different times during growth or early development are exposed to HS at 30°C for 30 min, a large increase in the levels

of 4-1 mRNA is observed, indicating that this cDNA encodes a Hsp. We refer to the 4-1 protein product as Hsp32.

Despite its low M_r , the deduced aa sequence of Hsp32 does not show any similarity with the small Hsp described in other organisms or with α -crystallins. In *Dd*, eight low- M_r proteins (26 to 32 kDa) have been shown to be HS induced, localized to the nuclei (Loomis and Wheeler, 1982); however, since these proteins were identified by radiolabeling cells under HS conditions and analysis, their identities, and thus their similarities to Hsp32, remain unknown. Further studies are in progress to characterize this novel *Dd* Hsp with respect to its localization and possible roles in growth, development and thermo-tolerance.

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