



Research paper

Cloning and analysis of *Dnaj* family members in the silkworm, *Bombyx mori*



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ABSTRACT

Heat shock proteins (Hsps) are involved in a variety of critical biological functions, including protein folding, degradation, and translocation and macromolecule assembly, act as molecular chaperones during periods of stress by binding to other proteins. Using expressed sequence tag (EST) and silkworm (*Bombyx mori*) transcriptome databases, we identified 27 cDNA sequences encoding the conserved J domain, which is found in *Dnaj*-type Hsps. Of the 27 J domain-containing sequences, 25 were complete cDNA sequences. We divided them into three types according to the number and presence of conserved domains. By analyzing the gene structures, intron numbers, and conserved domains and constructing a phylogenetic tree, we found that the *Dnaj* family had undergone convergent evolution, obtaining new domains to expand the diversity of its family members. The acquisition of the new *Dnaj* domains most likely occurred prior to the evolutionary divergence of prokaryotes and eukaryotes. The expression of *Dnaj* genes in the silkworm was generally higher in the fat body. The tissue distribution of *Dnaj1* proteins was detected by western blotting, demonstrating that in the fifth-instar larvae, the *Dnaj1* proteins were expressed at their highest levels in hemocytes, followed by the fat body and head. We also found that the *Dnaj1* transcripts were likely differentially translated in different tissues. Using immunofluorescence cytochemistry, we revealed that in the blood cells, *Dnaj1* was mainly localized in the cytoplasm.

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1. Introduction

Heat shock proteins (Hsps) are highly conserved, act as molecular chaperones during periods of stress by binding to other proteins. These proteins are involved in a variety of important biological functions, including protein folding, degradation, and translocation and macromolecule assembly (Lindquist, 1986). Hsps are divided into five major families based on their molecular weights as follows: Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100. *Dnaj* is the *Escherichia coli* homolog of the eukaryotic Hsp40 family and is an important molecular chaperone. *Dnaj* was first identified in *E. coli* as a 41-kDa heat shock protein (Georgopoulos et al., 1980). Its homologs have been identified in yeast, plants, animals, and humans. These homologs are characterized

by a highly conserved J domain, which is typically a 70-amino-acid N-terminal consensus sequence that facilitates interactions with Hsp70 family members (Kelley, 1999; Greene et al., 1998). *Dnaj* proteins possess at least four conserved domains: the J domain, a glycine- and phenylalanine-rich domain (termed G/F domain), a cysteine-rich zinc-finger domain (zf domain), and a less well-conserved C-terminal domain (C domain) that is thought to be involved in substrate binding. Although they are moderately conserved, the *Dnaj* homologs do vary in structure. *Dnaj* proteins can be divided into three subtypes (Qiu et al., 2006; Cheetham and Caplan, 1998). Type I *Dnaj* proteins contain the three conserved domains, the J domain, G/F domain, and zf domain. Type II proteins contain the J domain and the G/F domain. Type III proteins only possess the J domain. For example, human HDJ-2 (Chellaiah et al., 1993) and yeast YDJ-1 (Caplan and Douglas, 1991) contain the J, G/F, and zf domains; human HDJ-1 (Freeman et al., 1995) and yeast Sis1p (Lu and Cyr, 1998) contain the J and G/F domains; and human DNAJB6 (Menezes et al., 2012) and zutotin, which is a *Dnaj* molecular chaperone in yeast (Lu and Cyr, 1998), contain only the J domain. The J domain is typically located at the N terminus of the protein, but there are family members, such as yeast zutotin (Zhang et al., 1992), yeast Sec63 (Sadler et al., 1989), and human auxilin (Ahle and Ungewickell, 1990), in which it is found in the middle of the protein or at the C terminus.

Abbreviations: HSPs, Heat shock proteins; EST, expressed sequence tag; G/F domain, glycine- and phenylalanine-rich domain; zf domain, a cysteine-rich zinc-finger domain; C domain, C-terminal domain; RACE, random amplification of cDNA ends; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; *dnaJ*, gene encoding *E. coli* chaperonin; *CAJ1*, DnaK-like protein of yeast.

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Table 1
The gene family encoding DnaJ proteins in silkworm, *Bombyx mori*.

Gene	Related ESTs	Origin of mRNAs	Number of introns	cDNA length (ORF)	Protein length (amino acids)	Conserved domain (and amino acid positions)				GenBank accession no.	Location
						J	G/F	ZF	C		
<i>BmDnaJ1</i>	BP120274, BP120813, BP124909, BP181039, BP183135, BP183711, BP184291, CK525852, CK496195, CK500081, CK538632, CK538720, CK542922, CK539570, CK543848, CK488186, CK489441, CK492479, CK493676, CK515310, CK559960, CK560127, CK560152, CK563337, CK503582, BY922108, BB990679, BB988581, BJ984389, BJ983658, BB983021	Silk gland; ovary; whole body; pheromone gland; midgut; eye; fat body; maxilla; blood	1	1806 (28–1254)	408	7–56	74–107	141–196	112–338	FJ592074	Chr. 4
<i>BmDnaJ2</i>	AU005384, CK545739, CK485086, CK493585, CK517857, CK561914, CK563927, DY231295, DQ311436, BW999057, BB991794, NM_001046827	whole body; silk gland; ovary; uncharacterized tissue	≥7	2389 (177–1382)	401	6–57	75–100	150–204	109–335	FJ592075	Chr. 9
<i>BmDnaJ3</i>	BP182016, CK485689, CK530190, CN211993, BW997748, BY927645, BY928617, BY920199	testis; whole body; ovary; silk gland; pheromone gland	4	1202 (93–1154)	353	25–79	98–119		130–326	FJ592076	Chr. 4
<i>BmDnaJ4</i>	BP128110, BP128225, BP181517	testis; ovary	4	2380 (30–740)	236	3–58				FJ592077	Chr. 10
<i>BmDnaJ5</i>	BP179953, BP183588, CK518329, AB206400, CN374958, CN374959, BW997424, BY916124, NM_001043525	pheromone gland; silk gland; ovary; Malpighian tubule	2	1885 (117–1172)	351	4–57	74–120		173–337	FJ592078	Chr. 5
<i>BmDnaJ6</i>	BP128301, BP183667, CK527417, CK487909, CK486036, CK490992, CK562770, CK563960, CN379443, BY924089, BB993975, BB989143	silk gland; blood; testis; whole body; pheromone gland; Verson's gland	4	1970 (132–1181)	349	106–159				FJ592079	–
<i>BmDnaJ7</i>	BP177673, CK542314, CK485183, BY916941	Malpighian tubule; ovary; silk gland; prothoracic gland	1	1597 (177–785)	202	14–66				FJ592080	Chr. 15
<i>BmDnaJ8</i>	CK489761, CK560898, CK561341, BB987831	silk gland	0	1252 (146–1162)	338	36–> 68				FJ592081	Chr. 23
<i>BmDnaJ9</i>	AU002994, AV402116, AV402549, BP121428, BP125436, BP125599, BP181984, CK520631, CK527384, CK494359, CK538545, CK486078, CK487945, CK536077, CK514931, CK516529, CK517702, CK559412, CK560189, DQ311264, BY925833, BY917505, BY938190, BY938514, BY939058, BB982930, NM_001046720	silk gland; midgut; blood; ovary; fat body; eye; uncharacterized tissue	7	2596 (158–1642)	494	377–436				FJ592082	Chr. 4
<i>BmDnaJ10</i>	BP120578, BP121090, BP121472, CK520483, CK484976, CK489491, CK534758, CK516806, CK518288, CK558930, CK559634, CK564707, CK560511, CK562467, CK564185	testis; eye; silk gland; blood	4	1397 (188–997)	269	15–71				FJ592083	Chr. 9

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