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Phosphorylation of heat shock protein 40 (Hsp40/DnaJB1) by mitogen-activated protein kinase-activated protein kinase 5 (MK5/PRAK)

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ABSTRACT

Heat shock protein 40 (Hsp40) acts as a co-chaperone with Hsp70 to promote protein folding, protein transport and degradation. The human Hsp40 family contains more than 40 members, some of which can exist as phosphoproteins in the cell. However, little is known about the protein kinases responsible for their phosphorylation and the functional relevance of this post-translational modification remains elusive. Here we show that Hsp40/DnaJB1 is an *in vitro* and *in vivo* substrate for the mitogen-activated protein kinase-activated protein kinase 5 (MK5). MK5 and Hsp40/DnaJB1 form complexes in cells and this interaction is accomplished by the C-terminal regions of both proteins. MK5 can phosphorylate Hsp40/DnaJB1 at several residues *in vitro*. Studies with specific phosphoantibodies indicate that MK5 phosphorylates Hsp40/DnaJB1 *in vivo* at Ser-149 or/and Ser-151 and Ser-171 in the C-terminal domain of Hsp40/DnaJB1. MK5 modestly stimulates the ATP hydrolyse activity of Hsp40/Hsp70 complex and enhances the repression of heat shock factor 1 driven transcription by Hsp40/DnaJB1.

1. Introduction

Heat shock proteins (HSPs) are a group of structurally unrelated proteins dealing with cytotoxic stress (Ashburner and Bonner, 1979; Schlesinger, 1996; Hendrick and Hartl, 1993; Parsell and Lindquist, 1993). They are classified into six major families based on their relative molecular mass: HSP110/HSPH, HSP90/HSPC, HSP70/HSPA, HSP60/HSPD, HSP40/DNAJ and small heat shock proteins sHSP/HSPB. HSPs play a prime role in protein homeostasis by binding to substrates at risk, keeping them in a conformation either competent for refolding or degradation. As such, they belong to a much larger superfamily of chaperones (Ellis, 1987; Hartl and Hayer-Hartl, 2002, 2009; Vos et al., 2008; Kampinga et al., 2009; Garrido et al., 2012; Jackson, 2013).

More than 40 different human Hsp40/DnaJ proteins have been described so far. They are subdivided into types I, II and III based on the presence of conserved regions (Cyr et al., 1994; Cheetham and Caplan, 1998; Ohtsuka and Hata, 2000; Hennessy et al., 2000; Qiu et al., 2006; Hageman and Kampinga, 2009; Li et al., 2009). All types of Hsp40/DnaJ proteins contain the J domain, which consist of 70 amino acid residues that form four α-helices and encompass the conserved tripeptide of histidine, proline and aspartic acid (HPD motif; Fig. 1; Qian et al., 1996). This motif is involved in Hsp70 binding and is critical for stimulating Hsp70's ATPase activity by the J domain (Minami et al., 1996; Tsai and Douglas, 1996; Kampinga and Craig, 2010). In type I and II, the J domain is at the N-terminus, while it can be located at any position within the protein sequence in type III Hsp40 family members (Li et al., 2009). Type I and II Hsp40/DnaJs contain a glycine-/phenylalanine-rich region (G/F-region) whose function is ill defined, and type I also possesses a cysteine-rich stretch (four repeats of the CxxCxGxG type zinc finger) adjacent to the G/F-region. The C-terminal domain binds non-native polypeptides and then transfers them to Hsp70 (Langer et al., 1992; Hartl and Hayer-Hartl, 2002; Kampinga and Craig, 2010).

Hsp40/DnaJ members participate in processes such as protein translation, folding, unfolding, translocation and degradation. They perform their task by recruiting chaperone Hsp70 through their J domain and stimulating the ATPase activity of Hsp70. In general, Hsp70s display very low basal ATPase activity. A co-chaperone Hsp40/DnaJ protein can bind to substrate polypeptides by itself, and its J domain promotes ATP hydrolysis. Hsp70 refolds the non-native polypeptide using energy derived from ATP hydrolysis (Bukau and Horwich, 1998; Fan et al., 2003; Qiu et al., 2006; Kampinga and Craig, 2010). Hsp40/DnaJ proteins can also regulate

Abbreviations: CREB, cAMP response element binding protein; HSF, heat shock factor; HSP, heat shock protein; MK, mitogen-activated protein kinase-activated protein kinase; PK, protein kinase; PKA, cAMP-dependent protein kinase/protein kinase A.

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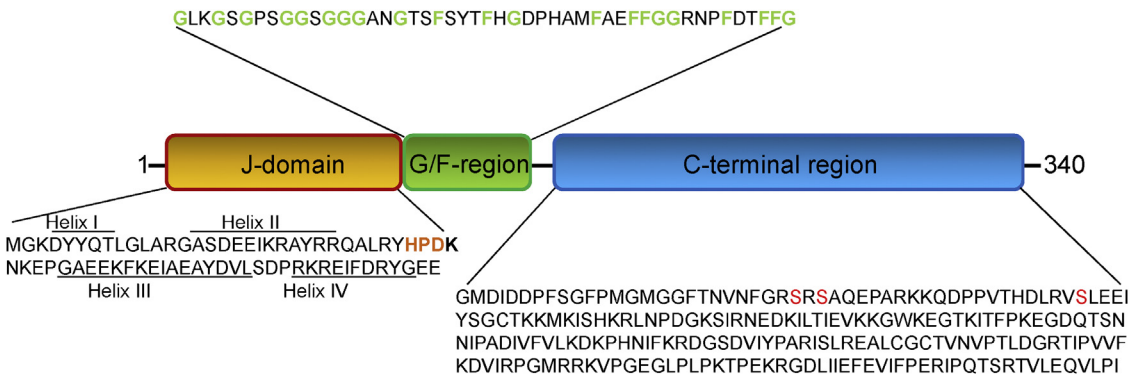

Fig. 1. Primary structural and functional domains of human DnaJB1 (GenBank accession number AK312624). The J-domain, the Gly/Phe-rich region (G/F-region) and C-terminal region and their respective amino acid composition are shown. The *in vivo* MK5 phosphoacceptor sites Ser-149, Ser-151 and Ser-171 are highlighted in red, and the G/F residues in the G/F region are shown in green. The four helices I–IV and the conserved HDP motif in the J-domain are indicated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

other chaperones, such as the 90-kDa heat shock protein Hsp90. The Hsp40/DnaJ protein TPR2 (DnaJC7) can mediate the retrograde transfer of substrates from Hsp90 onto Hsp70 (Brychzy et al., 2003).

Several studies have demonstrated that mammalian Hsps can be phosphorylated. Hsp27 can be phosphorylated by several protein kinases, including the protein kinases B (PKB/AKT), C (PKC), D (PKD), G (PKG), and the mitogen-activated protein-activated protein kinases MK2, MK3 and MK5 (reviewed in Kostenko and Moens, 2009). Hsp20 is phosphorylated at Ser-16 by cAMP-dependent protein kinase (PKA; Komalavilas et al., 2008; Edwards et al., 2012) and by cGMP-dependent kinase (Beal et al., 1997; Brophy et al., 2002), while Hsp90α can be phosphorylated by DNA-PK on Thr-5 and Thr-7 (Solier et al., 2012). Hsp70 is phosphorylated by polo-like kinase 1, while Bip is an *in vitro* Src kinase substrate (Carlino et al., 1994; Chen et al., 2011). Also phosphorylated forms of Hsp40/DnaJ proteins have been isolated, including DnaJA1, DnaJA3, DnaJA5, DnaJB4, DnaJB6, DnaJC1, DnaJC5, DnaJC6, DnaJC9, DnaJC12, DnaJC15 and DnaJC17 (Evans et al., 2001; Liu et al., 2005; Dephoure et al., 2008). DnaJC1 (=ERj1) can be phosphorylated *in vitro* by casein kinase-2, DnaJA3 (=TID1) is tyrosine-phosphorylated by Trk receptor tyrosine kinases, while DnaJC5 is phosphorylated by PKA on Ser-10 (Evans et al., 2001; Liu et al., 2005; Götz et al., 2009).

Mitogen-activated protein-activated protein kinase 5 (MK5/PRAK) is a serine/threonine kinase that is activated by the atypical mitogen-activated protein kinases ERK3 and ERK4 and by the conventional mitogen-activated protein kinase p38 (Schumacher et al., 2004; Seternes et al., 2004; Aberg et al., 2006; Kant et al., 2006). The activity of MK5 is also stimulated by cAMP-dependent protein kinase/protein kinase A (Gerits et al., 2007). Several MK5 substrates and interaction partners have been identified, including 14-3-3ε, Binder of Rho GTPase 2 and 3 (Borg 2 and 3), cell cycle and apoptosis regulator 1 (Ccar1), cytosolic phospholipase A2, eukaryotic initiation factor 2 (eIF2) kinase, transcription factors forkhead box-contain protein class O1 and 3a (FOXO1 and FOXO3a), Hsp27, DnaJ protein human tumorous imaginal disc1(S) ($hTid_S$), guaninenucleotide exchange factor Kalirin-7, E3-ubiquitin ligase Nedd4 (neural precursor cell expressed, developmentally down-regulated 4), p53, DNA repair protein RAD23B, the tyrosine kinase rearranged in transfection (RET), Ras homologue enriched in brain (Rheb), S100A calcium binding protein 11, scaffold attachment factor B2 (SAFB2), GTP-binding protein Septin 8, serine/arginine-rich splicing factor 1 (SFRS1), signal transduction protein small body size/mother against decapentaplegic-related protein 4 (SMAD4), transcriptional regulators staphylococcal nuclease domain containing 1 (SND1) and tripartite motif-containing 24 (TRIM24), tryptophane hydroxylase-2, tyrosine hydroxylase, ubiquitin-conjugating enzymes 9 (UBC9), 2I (UBE2I) and 2N (UBE2N), and the cytoskeletal protein zyxin. It has not been established whether all of them are genuine MK5 substrates, nor have the functional consequences of all these interactions been determined (Moens and Kostenko, 2013; Newman et al., 2013; Chow et al., 2013). MK5 phosphorylates Hsp27 at Ser-15, Ser-78 and Ser-82 and this posttranslational modification allows F-actin rearrangement. As such, the MK5-Hsp27 connection is involved in cell migration and metastasis (Tak et al., 2007; Kostenko et al., 2009; Stöhr et al., 2012). Because Hsp27 is a *bona fide* substrate of MK5, we wanted to determine whether other heat shock proteins are targets of MK5. We identified Hsp40/DnaJB1 as a novel MK5 substrate. MK5 phosphorylates Hsp40 *in vivo*, modestly stimulates the ATP hydrolase activity of the Hsp70/Hsp40 cycle, and increased Hsp40-mediated repression of transcriptional activity of the heat shock factor 1 (HSF1).

2. Materials and methods

2.1. Reagents

Recombinant Hsp27, GST-tagged Hsp40, GST-tagged Hsp70 and His-tagged Hsp90 were from SignalChem (Nordic Biosite AS, Oslo, Norway; cat. nos. H32-54G, H34-54G, and H36-50H, respectively). Active PKD1 and active MK5 were from Millipore (Billerica, MA, USA; cat. no. 14-508 and 14-334, respectively). Anti-flag M2 monoclonal antibody was from Aligent Technologies (Santa Clara, CA, USA; cat. no. 200471). Anti-green fluorescence protein (GFP) antibody (ab290) was purchased from AbCam (Cambridge, UK). Anti-Ras antibody was from Merck Millipore (RAS10, catalog number 05-516). The phosphospecific antibodies that recognize Hsp40 phosphorylated at serine residues 149 and 151 or at serine 171 were obtained from Genscript (Genscript, NJ, USA). These antibodies were generated by immunizing rabbits with the peptides GRpSRpSAQEPARKKQC and HDLRVpSLEEIYSGC, respectively. Secondary antibodies were purchased from Tropix (Applied Biosystems, Bedford, MA, USA). The protein marker MagicMark™ XP was from Life Technologies (Grand Island, NY, USA; cat. no. LC5602).

2.2. Plasmids

The plasmid pEGFP-C1 was obtained from Clontech Laboratories, Inc. (Mountain View, CA, USA). The Flag-Hsp40 plasmid was kindly provided by Dr. Atsushi Muraguchi (Ohtsuka, 1993; Liu et al., 2003). The expression plasmid cDNA/FRT/TO DNAJB1 was purchased from Addgene (Cambridge, MA, USA). The BamHI/NotI fragment of DNAJB1 was ligated into the