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Heat shock transcription factor HSF1 regulates the expression of the Huntingtin-interacting protein HYPK

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ABSTRACT

Background: The Huntingtin-interacting protein HYPK possesses chaperone-like activity. We hypothesized that the expression of HYPK could be regulated by heat shock factor HSF1, a transcriptional regulator of chaperone genes.
Methods: HYPK expression in HeLa cells was assessed by RT-PCR and Western blot analysis. In vivo binding of HSF1 to the *HYPK* promoter was analyzed by chromatin immunoprecipitation assays. The requirement for HYPK in heat-shocked cells was examined using HYPK-knockdown cells.
Results: Levels of *HYPK* mRNA were slightly increased by heat treatment; however, the levels decreased in HSF1-silenced cells. The *HYPK* promoter was bound by HSF1 in a heat-inducible manner; however, its core promoter activity was notably suppressed upon heat shock. When cells were exposed to heat shock, silencing *HYPK* caused a decrease in cell viability.
Conclusions: HYPK is a novel target gene of HSF1. HSF1 maintains HYPK expression in heat-shocked cells.
General significance: The maintenance of HYPK expression by HSF1 is necessary for the survival of cells under thermal stress conditions.

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1. Introduction

Molecular chaperones assist in the correct folding of nascent polypeptide chains and prevent the formation of non-specific protein aggregates [1,2]. Chaperone gene expression is primarily regulated by heat shock transcription factor HSF1, a central regulator of the heat shock response [3]. HSF1 is generally found in the cytoplasm as an inactive monomer. In response to proteotoxic stress, such as heat shock, heavy metals, and toxic substances, HSF1 undergoes transition from monomeric to trimeric state, becomes hyperphosphorylated, and localizes to the nucleus. Transcriptionally active HSF1 induces the expression of heat shock protein (HSP) genes through binding to the heat shock elements (HSEs) that consist of inverted repeats of the 5-bp sequence NGAAN. HSF1 is thus important in the maintenance of protein homeostasis and participates in the processes of aging, carcinogenesis, and protein-misfolding disorders [3–6].

Huntington's disease is an autosomal dominant neurodegenerative disorder caused by the expansion of polymorphic CAG trinucleotide repeats in the 1st exon of the Huntingtin gene (*HTT*). The CAG repeats are translated into a polyglutamine (polyQ) stretch, and the polyQ expansion in the Htt protein results in conformational changes leading to the formation of aggregates in cells [7–9]. Htt polyQ-interacting proteins, including HYPK (Huntingtin yeast two-hybrid protein K), have been identified by yeast two-hybrid screens [10,11].

The biological importance of HYPK is shown by the observation that knockdown of *HYPK* expression in human cell lines is associated with cell cycle arrest and apoptosis [12,13]. In cellular models of Huntington's disease, HYPK reduces Htt polyQ aggregation upon overexpression [14], whereas knockdown of *HYPK* results in increased aggregation [12]. HYPK also reduces the heat-induced aggregation of cellular proteins and enhances the recovery of heat-denatured or unfolded proteins [13,14]. HYPK copurifies with the ribosome-associated complex [12,15]. HYPK interacts with the N^{α}-terminal acetyltransferase (NatA) complex, which is responsible for cotranslational N-terminal acetylation of the nascent polypeptide on the ribosome, and is required for efficient N-terminal acetylation of a known NatA substrate [12]. Interactions between HYPK and other proteins have been observed, which play roles in protein folding, response to unfolded proteins, cell cycle arrest, anti-apoptosis pathways, and transcriptional regulation [13]. These observations suggest that HYPK possesses chaperone-like activity [12–14].

In this report, we analyzed whether *HYPK* is a target of HSF1. Our observations showed that HSF1 was necessary to maintain HYPK expression in heat-shocked cells. We discuss the impact of the transcriptional regulation of *HYPK* by HSF1.

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2. Materials and methods

2.1. Plasmids

The promoter fragment of *HYPK* (from −2328 to +530, relative to the translation initiation site) was cloned upstream of the firefly luciferase gene of the pGL3-Basic vector (Promega) to create *HYPK*(−2328)-*LUC*. Deletion and nucleotide substitution mutations were introduced using standard methods. The plasmid HSE3P-SV40p-*LUC* contained an HSE oligonucleotide of three inverted NGAAN repeats upstream of the SV40 promoter in the pGL3-Promoter vector (Promega) [16]. The SV40 promoter region was replaced with the *HYPK* promoter fragments (−94 to +530 and −47 to +530) to create HSE3P-*HYPK*p(−94)-*LUC* and HSE3P-*HYPK*p(−47)-*LUC*, respectively. In plasmid *HYPK*$_{HSE}$-SV40p-*LUC*, the *HYPK* HSE fragment (−170 to −76) was inserted upstream of the SV40 promoter in the pGL3-Promoter vector. Plasmid *HYPK*$_{HSE}$-SV40p-IVS-*LUC* contained the chimeric intron of pRL-TK (Promega) between the SV40 promoter and firefly luciferase gene of *HYPK*$_{HSE}$-SV40p-*LUC*.

Plasmid pBK266 was a derivative of pcDNA3.1(+) (Invitrogen) containing three copies of the influenza hemagglutinin (HA) tag sequences between the EcoRI and XbaI sites. The *HYPK* fragment from −306 (AatII site) to +1205 (an EcoRI site was created by PCR) contained the 5′ promoter region, the 1st to 3rd exons, the 1st to 3rd introns, and the 3′ terminus of the HYPK coding region in the 4th exon. This fragment was cloned into the AatII and EcoRI sites of pBK266; in this plasmid (pBK267), the cytomegalovirus promoter of pBK266 was deleted, and the C-terminus of HYPK was fused in-frame to the HA tag. Plasmid pBK273 contained nucleotide substitution mutations in the *HYPK* HSE of pBK267.

The stably replicating shRNA expression vector pBK192 contained the human H1 promoter, the EBNA1-OriP replication system, and a puromycin resistance gene [17]. In plasmids pBK195 and pBK279, HSF1 shRNA and HYPK shRNA, respectively, were expressed under the control of the H1 promoter (Supplementary Table S1).

2.2. Cell culture and transfection

HeLa cells were maintained as described previously [18,19]. Transfection was carried out using HilyMax reagent (Dojindo Laboratories). Cells harboring pBK192 (empty), pBK195 (shHSF1), or pBK279 (shHYPK) were cultured in the presence of 1.0 μg/ml puromycin.

2.3. RT-PCR assay

Total RNA was isolated from cells and analyzed by RT-PCR as described previously [18,19]. The primer sequences are listed in Supplementary Table S1. The PCR product quantities were quantified using GelPro Analyzer software (Media Cybernetics) and were compared after normalizing each sample to the amounts of *ACTB* (β-actin) and 18S rRNA PCR products.

2.4. Luciferase assay

HeLa cells cultured in 24-well plates were transfected with DNA mixtures containing firefly luciferase reporter plasmid and *Renilla* luciferase control plasmid (pRL-TK). The plasmids expressing constitutively active HSF1 were HSF1-VP, containing the HSF1-VP16 activation domain fusion construct [16], and HSF1-ΔR, containing the HSF1 construct lacking the central repression domain [20]. For heat shock experiments, the cells were exposed to 42.5 °C for 1 h and then recovered at 37 °C for 4 h. The luciferase assay was performed using the Dual-Luciferase Reporter Assay System (Promega), as described previously [18,19].

2.5. Chromatin immunoprecipitation assay

The chromatin immunoprecipitation assay was carried out as described previously [18,19]. The cross-linked chromatin was immunoprecipitated by an anti-HSF1 antibody (kindly provided by Dr. Akira Nakai) and control IgG. Input and immunoprecipitated samples were analyzed by PCR using the primers listed in Supplementary Table S1.

2.6. Western blot analysis

Cell extracts were prepared and subjected to Western blot analysis using anti-HYPK (Sigma), anti-HSF1, anti-HA (Sigma), and anti-GAPDH (Sigma) antibodies [16,18].

2.7. Statistical analysis

The data are representative of at least three independent experiments. Significant differences were analyzed by Student's *t*-test.

3. Results

3.1. HSF1 is involved in transcriptional regulation of HYPK

To analyze whether *HYPK* mRNA levels change under thermal stress conditions, HeLa cells were exposed to heat shock at 42.5 °C for 1 h and were then allowed to recover at 37 °C for 0, 1, 2, 4, or 6 h. RT-PCR was conducted using primers for *HYPK*, heat-inducible *HSC70*, *HSP70*, and *HSP90*, and constitutively expressed *ACTB* (Fig. 1A). The mRNA levels of *HYPK* gradually accumulated and reached a 1.8-fold increase at 6 h of recovery after heat shock. The *HSC70* mRNA levels modestly increased, peaking at 4 h of recovery. The expression of *HSP70* and *HSP90* was strongly induced under the heat shock conditions. Therefore, the *HYPK* mRNA levels increase after heat shock; however, the time course of induction is different from that of *HSC70*, and the levels of induction are significantly lower than those of *HSP70* and *HSP90*. We also analyzed levels of HYPK protein using an anti-HYPK antibody and found that the levels remain almost constant during heat shock and subsequent recovery (Fig. 1B). The localization of HYPK protein in heat-shocked cells was examined by immunostaining of cells expressing HA-tagged HYPK (Supplementary Fig. S1). The results showed that HYPK-HA localized mainly to the cytoplasm (although some staining was observed in the nucleus) and that heat shock did not notably affect the localization of the protein.

The involvement of HSF1 in *HYPK* expression was examined using HSF1-knockdown cells. HSF1 protein levels in cells transfected with the HSF1 shRNA expression plasmid were reduced to less than 15% of those in control cells transfected with empty vector plasmid (Fig. 1C). Silencing HSF1 did not significantly affect constitutive *HYPK* mRNA levels under non-heat shock conditions (Fig. 1D). However, the *HYPK* mRNA levels in HSF1-silenced cells did not increase but instead decreased to 80% at 3 h of recovery after heat shock. These results suggest that HSF1 is necessary to maintain *HYPK* mRNA levels under thermal stress conditions.

3.2. Identification of HSE in the HYPK promoter

The *HYPK* promoter region from −2328 to +530 relative to the translation initiation site was cloned upstream of the luciferase gene to construct *HYPK*(−2328)-*LUC* (Fig. 2). A fusion protein composed of full length HSF1 and the herpes simplex virus VP16 activation domain (HSF1-VP) has been shown to function as a potent activator of HSE-containing genes under non-heat shock conditions [16]. Co-transfection of *HYPK*(−2328)-*LUC* with HSF1-VP was associated with a 2.5-fold increase in luciferase activity, suggesting the presence of an HSE in the *HYPK* promoter. When cells transfected with the reporter

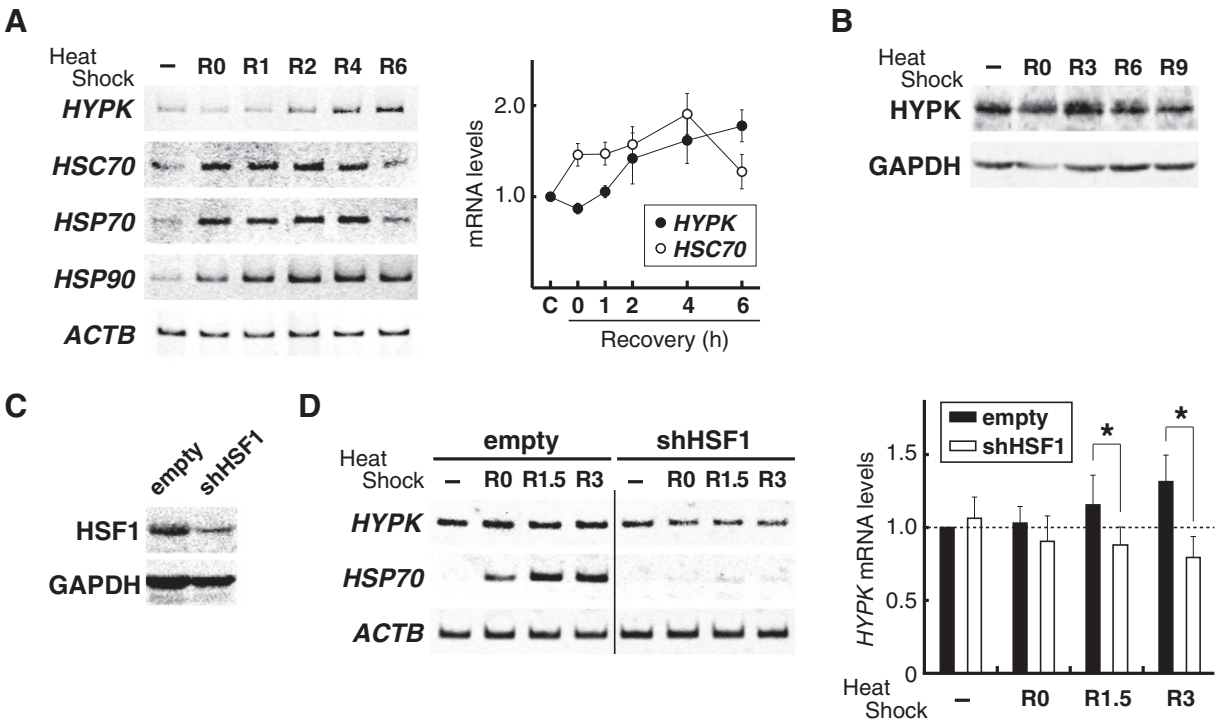

Fig. 1. Transcriptional regulation of *HYPK* by HSF1. (A) *HYPK* mRNA levels in heat-shocked cells. HeLa cells cultured at 37 °C (−) were heat-shocked at 42.5 °C for 1 h and recovered (R) at 37 °C for 0, 1, 2, 4, or 6 h. Total RNA prepared from the cells was subjected to RT-PCR. The relative mRNA levels of *HYPK* and *HSC70* compared with non-heat-shocked control (C) are expressed as the mean ± standard deviation of three independent experiments. (B) HYPK protein levels in heat-shocked cells. Cells cultured at 37 °C (−) were heat-shocked at 42.5 °C for 1 h and recovered (R) at 37 °C for 0, 3, 6, or 9 h. Protein extracts were prepared and subjected to Western blotting with anti-HYPK and anti-GAPDH antibodies. (C) HSF1 protein levels in HSF1-silenced cells. Cells transfected with an empty vector plasmid or an shHSF1 expression plasmid were cultured in medium supplemented with 1.0 μg/ml puromycin. Cell extracts were subjected to Western blotting with anti-HSF1 and anti-GAPDH antibodies. (D) *HYPK* mRNA levels in HSF1-silenced cells. Cells were cultured in puromycin-free medium for at least 15 h at 37 °C (−) and were then heat-shocked at 42.5 °C for 1 h and allowed to recover (R) at 37 °C for 0, 1.5, or 3 h. Total RNA prepared from the cells was subjected to RT-PCR. The relative *HYPK* mRNA levels compared with empty vector cells cultured at 37 °C are expressed as the mean plus standard deviation of three independent experiments. $^{*}p < 0.05$.

gene alone were heat-shocked at 42.5 °C for 1 h, the luciferase activity increased 1.3-fold.

Successive deletions from the 5′ region in *HYPK-LUC* showed an essential role for the region from −170 to −95 in the response to HSF1-VP (Fig. 2). In heat-shocked cells, the removal of this region resulted in a decrease, rather than an increase, in luciferase activity. This region contains an HSE-like sequence, −114 C**T**C**C**CT**GAA**GC**TTC**TA**GAA**C −94 (matched nucleotides are shown in bold letters), consisting of four NGAAN inverted repeats. The expression of the *HYPK*(HSEm)-*LUC* reporter containing nucleotide

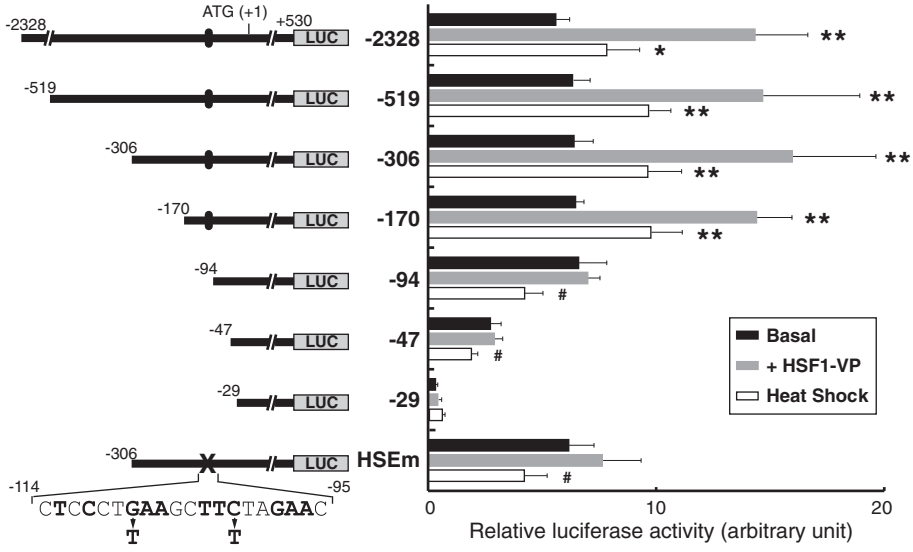

Fig. 2. Expression of *HYPK* promoter-luciferase reporters. The structures of the *HYPK-LUC* reporter genes are shown on the left. Thick lines with numbers indicate the *HYPK* promoter and the nucleotide positions. Ovals represent the HSEs. In *HYPK*(HSEm)-*LUC*, the nucleotide sequence of the HSE has been changed (x) as shown below. The results of the luciferase assay are shown on the right. Firefly luciferase activities (arbitrary units) are calculated after normalization to *Renilla* luciferase values and are expressed as the mean plus standard deviation of four experiments. Asterisks and hash marks indicate statistically significant upregulation and downregulation, respectively, by HSF1-VP or heat shock compared with uninduced (basal) controls ($^{*}p < 0.05$; $^{**}p < 0.01$; $\#p < 0.05$).

substitutions in the HSE-like sequence was not induced by HSF1-VP and was inhibited by heat shock. Therefore, this sequence functions as an HSE in the *HYPK* promoter. It should be noted that basal-level expression of *HYPK* was maintained by the region from −94 to −30.

3.3. Characterization of the HSE and promoter

The binding of HSF1 to the *HYPK* promoter was analyzed by chromatin immunoprecipitation assays. As shown in Fig. 3A, HSF1 bound to the *HYPK* promoter (−150) under non-heat shock conditions, and the binding was further induced by heat shock. The specificity was indicated by the lack of HSF1 binding to the *HYPK* 3′ region (+1100). In electrophoretic mobility assays, HSF1 bound to the *HYPK* HSE in a heat-inducible manner (Supplementary Fig. S2).

Although the *HYPK* promoter contains the HSE, the levels of the heat shock response of *HYPK* were much lower than those of *HSP70* and *HSP90* (see Fig. 1). We investigated the roles of the HSE ($HYPK_{HSE}$ from −170 to −85) and the core promoter (*HYPK*p from −94 to +530) in transcriptional regulation by HSF1. To explore the effect of HSF1 under non-heat shock conditions, we used a constitutively active HSF1 (HSF1-ΔR) that lacks the central repression domain [20]. As shown in Fig. 3B, the luciferase activity of *HYPK*(−170)-*LUC* was increased 2.2- and 1.5-fold by HSF1-ΔR and heat treatment, respectively. When $HYPK_{HSE}$ was placed upstream of the SV40 promoter (SV40p), the chimeric construct $HYPK_{HSE}$-SV40p-*LUC* responded well to HSF1-ΔR and heat shock, compared with the induction via a synthetic canonical HSE (HSE3P) in HSE3P-SV40p-*LUC*. Therefore, the *HYPK* HSE, similar to HSE3P, efficiently mediates HSF1 activity. The expression of HSE3P-*HYPK*p(−94)-*LUC*, in which $HYPK_{HSE}$ was replaced with HSE3P, was induced by HSF1-ΔR and heat shock to a similar extent as *HYPK*(−170)-*LUC*. Notably, robust induction by HSF1-ΔR was observed when HSE3P was positioned close to the core promoter in HSE3P-*HYPK*p(−47)-*LUC*. It is known that as the distance between the activator and the promoter decreases, the activator function improves [21]. In comparison with HSF1-ΔR, heat shock had only a small effect on the expression of the reporter ($p = 1.8 \times 10^{-4}$). The removal of the HSE in *HYPK-LUC* was associated with a decrease in the luciferase activity in heat-shocked cells (see Fig. 2). Therefore, although the *HYPK* HSE is able to mediate the activation signal of HSF1, the core promoter activity is heat-sensitive, thereby responding to heat shock at low levels.

3.4. Expression of HYPK under various stress conditions

The expression of *HYPK* in cells exposed to various stress-inducing agents was analyzed by RT-PCR (Fig. 4A). Celastrol, a traditional Chinese medicine, is known to down-regulate the HSP90 activity. MG132 is a peptide aldehyde inhibitor of the proteasome. Both agents induce the heat shock response and activate HSF1 [19,22–24]. In cells treated with celastrol or MG132, the mRNA levels of *HSP70* were robustly increased (>3.5-fold), and those of *HSC70* were slightly increased (~1.5-fold). However, these agents did not significantly induce the expression of *HYPK*. We then tested HSF1 occupancy of the *HYPK* promoter. The results of chromatin immunoprecipitation assays showed celastrol- and MG132-induced binding of HSF1 (Fig. 4B). When the *HYPK* HSE was placed upstream of the SV40 promoter, the luciferase mRNA levels of the chimeric construct $HYPK_{HSE}$-SV40p-IVS-*LUC* were increased in cells treated with celastrol or MG132 (Fig. 4C). Therefore, the *HYPK* HSE is able to mediate celastrol- and MG132-induced transcriptional activity of HSF1. The activity of the *HYPK* core promoter under the stress conditions was analyzed using the *HYPK*(−94)-*LUC* reporter (Fig. 4D). Both celastrol and MG132 did not alter luciferase mRNA levels, suggesting that unlike heat treatment these agents do not inhibit the core promoter activity of *HYPK*. It remained unknown why the *HYPK* core promoter failed to respond to celastrol- and MG132-induced HSF1.

In the oxidative stress response, reactive oxygen species such as hydrogen peroxide have been suggested to induce HSF1 trimerization via formation of inter-molecular disulfide bonding between HSF1 monomers, thereby activating HSF1 [25]. In our hands, however, hydrogen peroxide did not significantly alter *HYPK* or *HSP70* mRNA levels (Fig. 4A). Tunicamycin inhibits protein glycosylation in the endoplasmic reticulum and induces the unfolded protein response [26]. The mRNA levels of *HYPK* and *HSP70* remained unchanged in cells treated with this drug. Of note, induction of the oxidative stress and the unfolded protein response was verified by an increase in *ATF3* mRNA levels. As shown in Fig. 4B and D, these agents did not affect HSF1-*HYPK* promoter interaction or the *HYPK* core promoter activity. These results suggest that transcription of *HYPK* is not regulated by hydrogen peroxide or tunicamycin.

It has been shown that transcription of *HSC70* and *HSP70* is induced by serum stimulation, presumably independently of HSF1 [27,28]. We examined the mRNA levels of *HYPK* in cells that were serum-starved and subsequently serum-stimulated. As shown in Fig. 4A, *HYPK*

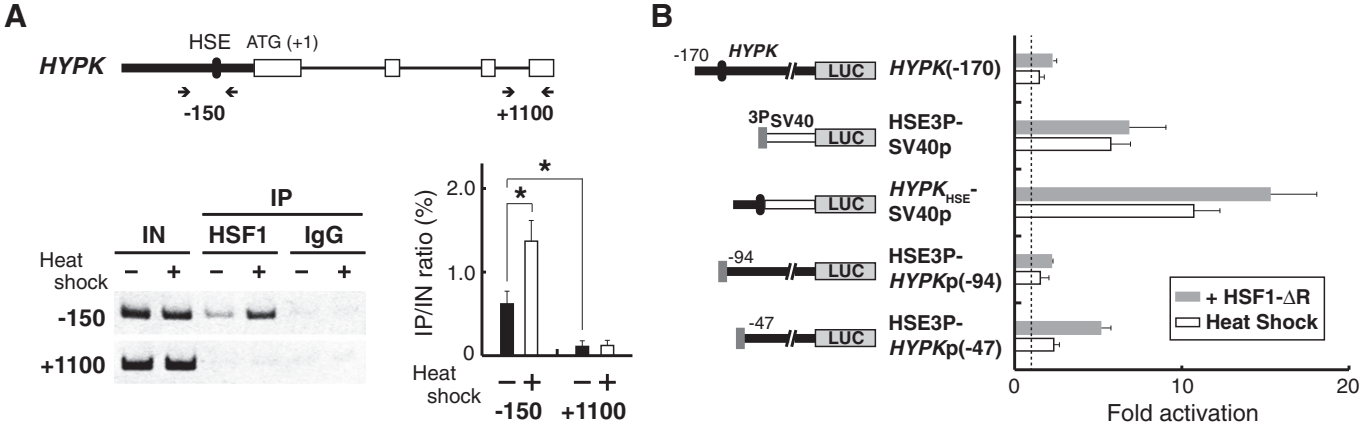

Fig. 3. Analysis of *HYPK* promoter. (A) Binding of HSF1 to the *HYPK* promoter. The upper panel shows the positions of the primers used for the chromatin immunoprecipitation assays. Thick line and oval indicate the *HYPK* promoter and HSE, respectively. Open boxes with thin lines represent exons and introns. Arrows show the positions of the primers. The lower panel shows the results of the chromatin immunoprecipitation analysis. Cells cultured at 37 °C were heat-shocked at 42.5 °C for 40 min. Chromatin samples were subjected to immunoprecipitation with an anti-HSF1 antibody and control IgG. DNA fragments prepared from input samples (IN) and immunoprecipitates (IP) were analyzed by PCR. The IP/IN ratios are expressed as the mean plus standard deviation of three independent experiments. *$p < 0.05$. (B) Expression of chimeric luciferase reporters. Gray and white boxes indicate the synthetic HSE (3P) and SV40 promoter, respectively. Firefly luciferase activities (− fold activation) are calculated relative to those of uninduced cells and are expressed as the mean plus standard deviation of four experiments.

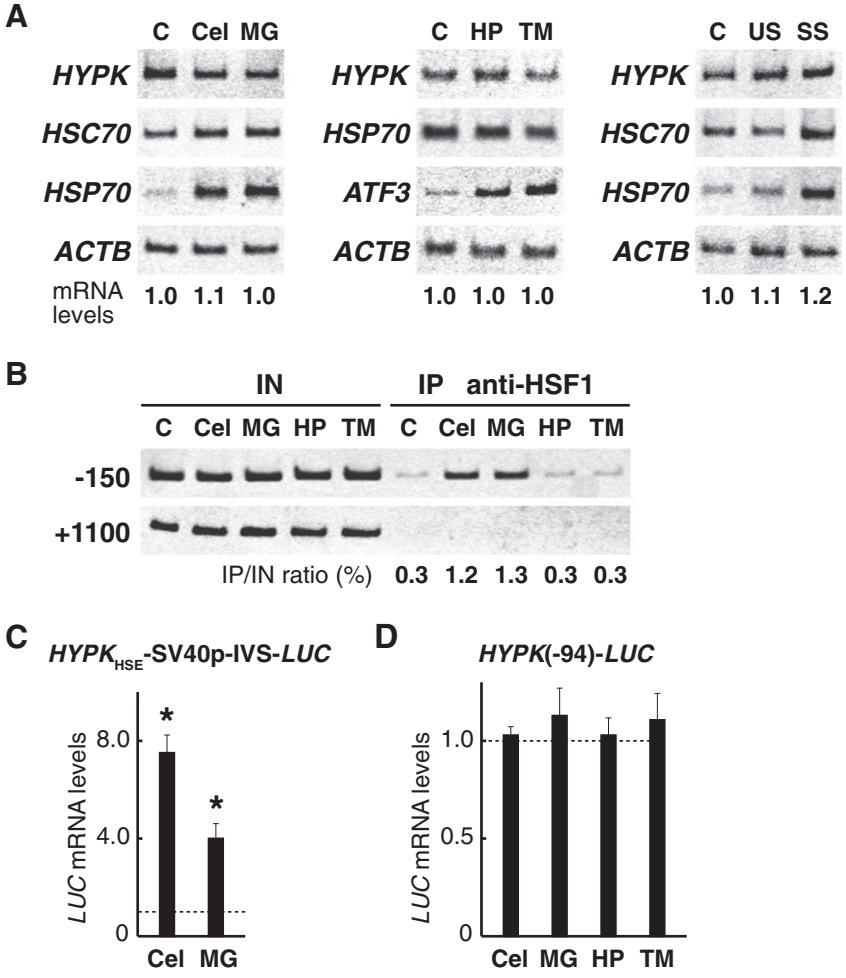

Fig. 4. Expression of *HYPK* in cells exposed to various stresses. (A) *HYPK* mRNA levels. Cells cultured at 37 °C (C) were treated with 5.0 μM celastrol (Cel), 5.0 μM MG132 (MG), 0.1 mM hydrogen peroxide (HP), or 2.5 μg/ml tunicamycin (TM) for 4 h. For serum stimulation, cells were cultured in medium without fetal bovine serum for 48 h and were then stimulated with 20% serum for 15 h (SS), or were left unstimulated (US). Total RNA prepared from the cells was subjected to RT-PCR. The relative levels of *HYPK* mRNA are shown below. (B) Binding of HSF1 to the *HYPK* promoter. Cells were treated with stress-inducing agents for 1.5 h. Chromatin samples were subjected to immunoprecipitation with an anti-HSF1 antibody, and input samples (IN) and immunoprecipitates (IP) were analyzed by PCR. The IP/IN ratios of the *HYPK* promoter region (−150) are shown below. (C) *HYPK* HSE activity. Cells transfected with *HYPK*$_{HSE}$-SV40p-IVS-*LUC* were trypsinized and divided into wells; the cells were then treated with stress-inducing agents for 4 h, or were left untreated. Total RNA was subjected to RT-PCR with SV40p-IVS-*LUC* and *ACTB* primers. The relative *HYPK*$_{HSE}$-SV40p-IVS-*LUC* mRNA levels compared with untreated cells are expressed as the mean plus standard deviation of three independent experiments. $^{*}p < 0.05$. (D) *HYPK* promoter activity. Cells were transfected with *HYPK*(−94)-*LUC*, divided into wells, and treated with stress-inducing agents for 4 h. Total RNA was subjected to RT-PCR with *HYPK-LUC* and *ACTB* primers. The relative *HYPK*(−94)-*LUC* mRNA levels compared with untreated cells are expressed as the mean plus standard deviation of three independent experiments.

expression remained unchanged in serum-starved and serum-stimulated cells, although the expression of *HSC70* and *HSP70* was induced after serum addition.

3.5. HYPK is necessary for resistance to heat stress

The *HYPK*-HA construct containing the HA-tag sequences at the 3′ end of the open reading frame was introduced into cells, and the expression of the HYPK-HA protein was analyzed by Western blotting with an anti-HA antibody (Fig. 5A). Consistent with the results of the luciferase assay, HYPK-HA levels slightly increased in heat-shocked cells; however, mutations in the *HYPK* HSE caused a decrease, rather than an increase, in HYPK-HA levels. Taken together with the results of mRNA analysis, these findings show that the HSF1-HSE interaction is necessary for the maintenance of HYPK protein in heat-shocked cells.

To explore the requirement for HYPK protein in heat-shocked cells, *HYPK* shRNA was used. Although *HYPK* is necessary for cell viability [6,7], *HYPK* mRNA levels in the shHYPK cells decreased to only 60% of the levels in control empty vector cells (Fig. 5B), and the growth rate of shHYPK cells was comparable to that of control cells at 37 °C (Fig. 5C). The cells were exposed to 42.5 °C for 90 min, and the viable cell number was determined by trypan blue staining after 24 h of recovery at 37 °C (Fig. 5C). Although the number of non-heat-shocked control cells increased 2.1-fold, the number of heat-shocked cells did not change (1.0-fold) without increasing the number of trypan blue-positive (dead) cells. These results suggest that the cells stopped proliferating after the heat challenge. The number of heat-shocked shHYPK cells decreased to 76% of the initial cell number, which was 33% of that of the non-heat-shocked control. Therefore, a reduction in the expression of HYPK results in a higher temperature sensitivity of the cells.

4. Discussion

Constitutive expression of the chaperone-like protein HYPK was slightly upregulated by heat shock stress, but not by oxidative stress or endoplasmic reticulum stress. Consistently, the *HYPK* promoter contained the HSE and was bound by HSF1 in a heat-inducible manner. In heat-shocked cells, reduced expression of *HYPK* caused a decrease in

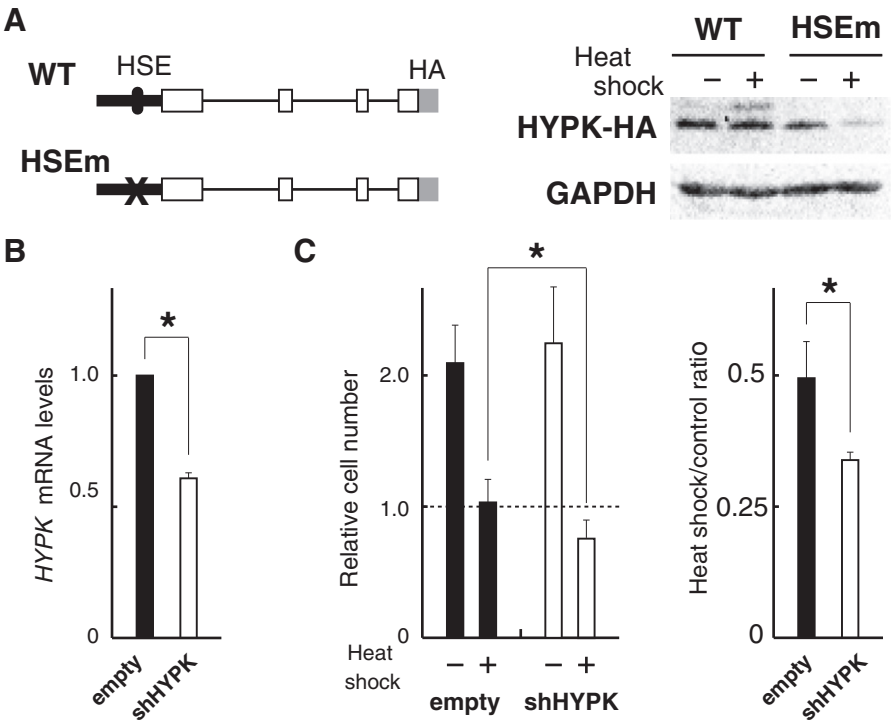

Fig. 5. Roles of HYPK protein in heat-shocked cells. (A) Expression of HYPK-HA in heat-shocked cells. The left panel shows the structures of the HYPK-HA expression constructs. The wild-type construct (WT, pBK267) contains the HSE (oval), whereas the mutant construct (HSEm, pBK273) lacks the HSE (x). Gray boxes represent the HA epitope. The right panel shows the results of Western blot analysis. Cells transfected with the indicated HYPK-HA constructs were trypsinized and plated into two wells; after 18 h, one well was heat-shocked at 42.5 °C for 1.5 h and then recovered at 37 °C for 4 h; the other well was untreated. Cell extracts were subjected to Western blotting with anti-HA and anti-GAPDH antibodies. (B) *HYPK* mRNA levels in shHYPK cells. Cells transfected with an empty vector plasmid or an shHYPK expression plasmid were cultured in medium supplemented with 1.0 μg/ml puromycin. Total RNA prepared from the cells was subjected to RT-PCR with *HYPK* and 18S rRNA primers. The relative *HYPK* mRNA levels compared with empty vector cells are expressed as the mean plus standard deviation of three independent experiments. $^*p < 0.05$. (C) Heat sensitivity of shHYPK cells. Cells were cultured in puromycin-free medium for at least 15 h at 37 °C, and viable cells were counted using the trypan blue exclusion method. Cells were heat-shocked at 42.5 °C for 90 min or were left untreated. After 24 h, viable cells were counted. The left panel shows the relative cell number compared with day 0. The right panel shows the ratio of the cell numbers of heat-treated versus untreated cells. The data are expressed as the mean plus standard deviation of three independent experiments. $^*p < 0.05$.

cell viability. We suggest that HSF1-mediated HYPK expression is necessary for cell survival under thermal stress conditions.

HSF1 is known as a strong transcriptional activator of HSP genes [3]. However, the levels of the heat shock response of *HYPK* were much lower than those of *HSP70* and *HSP90*. Constitutively expressed *HSC70*, which contains the HSE, was moderately induced by heat shock. The accumulation of *HYPK* mRNA was delayed relative to that of *HSC70* mRNA. Although the *HYPK* HSE serves as a potent *cis*-acting element, the *HYPK* promoter is sensitive to heat, and the mRNA levels do not increase immediately after heat shock. Low level transcriptional activation by HSF1 contributes to the maintenance of HYPK protein levels in heat-shocked cells. The cellular role of HYPK is implicated in protein maturation [12,15]. Heat shock transiently attenuates protein synthesis [29]. It is possible that sufficient levels of HYPK protein are necessary to resume translation.

The *HYPK* promoter lacks an apparent TATA sequence. In yeast, many stress-inhibited genes are general housekeeping genes and often lack a TATA sequence [30]. In mammalian cells, heat shock inhibits the activity of RNA polymerase II and represses transcription; however, how the heat-inducible genes overcome the repression remains unknown [31]. In addition, the *HYPK* core promoter failed to respond to the activation signal of celastrol- and MG132-induced HSF1. Activators are functionally linked to core promoters, and some activators prefer TATA-containing promoters, whereas others prefer TATA-less promoters [32,33].

HYPK possesses chaperone-like activity [12–14]. We have shown that HSF1 maintains HYPK expression in heat-shocked cells and that HYPK is necessary for resistance to heat stress. The forced expression of constitutively active HSF1 leads to an increase in the promoter activity of *HYPK*. We propose that active HSF1 induces the expression of HYPK and other target proteins, including various chaperones, and ameliorates protein misfolding and aggregation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2013.12.006.

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