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ORIGINAL PAPER

Heat shock factor 4 regulates the expression of HSP25 and alpha B-crystallin by associating with DEXD/H-box RNA helicase UAP56

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Abstract

Heat shock factor 4 controls the transcription of small heat shock proteins (e.g., HSP25, alpha B-cyrstallin, and r-crystallin), that play important roles in modulating lens proteostasis. However, the molecular mechanism underlying HSF4-mediated transcription is still unclear. Using yeast two hybrid, we found that HSF4 interacts with the ATP-dependent DEXD/H-box RNA helicase UAP56, and their interaction in lens epithelial cell line was further confirmed by GST-pull down assay. UAP56 is a vital regulator of pre-mRNA splicing and mature mRNA nuclear export. The immunofluorescence assay showed that HSF4 and UBA56 co-localize with each other in the nucleus of lens epithelial cells. Ectopic UAP56 upregulated HSF4-controlled HSP25 and alpha B-crystallin proteins expression, while knocking down UAP56 by shRNA reversed it. Moreover, UAP56 interacts with and facilitates the nuclear exportation of HSP25 and alpha B-crystallin mRNA without impacting their total mRNA expression level. In lens tissues, both UAP56 and HSF4 are expressed in the same nucleus of lens fiber cells, and their expression levels are simultaneously reduced with fiber cell maturation. Taken together, these data suggested that UAP56 is a novel regulator of HSF4 and might upregulate HSF4's downstream mRNA maturation and nuclear exportation.

Keywords HSF4 · UAP56 · HSP25 · Alpha B-crystallin · Posttranscriptional modification

Introduction

Lens development is regulated by temporospatial activation and inactivation of a number of transcriptional factors (Kondoh 1999). HSF4-orchestrated heat shock response, rather than HSF1 or HSF2, is indispensible for ocular lens development (Fujimoto et al. 2004). Genetic mutations in the HSF4 DNA-binding domain are closely associated with hereditary autosomal dominant cataracts (Bu et al. 2002). Knocking down HSF4 causes postnatal cataracts in the mouse model (Fujimoto et al. 2004). Thus, the role of HSF4 in fine-tuning the expression of specific target genes is important in maintaining homeostasis during lens development.

HSF4 transcriptional activity is essential in modulating proteostasis in postnatal lens tissue (Nakai et al. 1997). In the Hsf4-knock out lens tissue, the fiber cells are injured by the accumulation of aggregated proteins and delayed nuclear removal (Fujimoto et al. 2004; Min et al. 2004). In vitro data suggested that HSF4 could regulate FGF2-induced morphology transition from epithelial cells to fiber cells (Hu et al. 2013), protect the cells from stress-induced apoptosis, modulate lysosomal pH and hydrolytic activity (Cui et al. 2016), and regulate DNA injury repair (Cui et al. 2012). These functions are associated with its downstream targets (e.g., small heat shock proteins HSP25 and alpha B-crystallin or RAD51). HSF4 drives the transcription of its target genes by binding to the HSE elements in the promoters. The chromatin remodelers

Highlight 1. HSF4 interacts with UAP56, and they co-localize in the nucleus of lens epithelial cell line in vitro.
2. UAP56 is recruited by HSF4 to upregulate the nuclear exportation of HSP25 and alpha B-crystallin mRNAs.
3. UAP56 expresses developmentally in the postnatal lens, and its expression pattern in postnatal cortical fiber nucleus is similar to HSF4.

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BRG1, H3K4 trimethylation, and MAPKase are involved in regulating HSF4's transcription activity (He et al. 2010; Hu and Mivechi 2006; Tu et al. 2006). Accumulating evidence suggests that transcriptional RNA synthesis, pre-mRNA splicing, and nuclear export are coupled together (Proudfoot et al. 2002). However, the regulatory mechanism between HSF4 and its downstream pre-mRNA processing still remains unclear.

UAP56 (also known as BAT1) is an ATP-dependent DEXD/H-box RNA helicase that belongs to the U2 RNA helicase superfamily (Fleckner et al. 1997). UAP56 contains two DEXD/H-box regions at both the N- and C-termini that are linked by a flexible middle region. UAP56 binds and hydrolyzes ATP and unwinds the DsRNA through its dsRNA helicase activities (Shen et al. 2008). UAP56 forms the different spliceosome complex E, B, and C by associating with U2AF65, U4, and U6, respectively, and participates in the pre-RNA splicing processes (Luo et al. 2001). UAP56 is also an important component of the TREX complex through interacting with Aly, CIP29, and THO. This complex regulates mRNA synthesis, splicing, and nuclear export (Li et al. 2005). UAP56 is regulated by PLK1 kinase phosphorylation (Xiong et al. 2012). Recently, UAP56 has been reported to interact with BRC (Sahni et al. 2012), upregulating the E2F transcription activity, DNA synthesis, and vascular smooth muscle cell proliferation. In this paper, we found that HSF4 interacted with UAP56 in yeast two hybrid and lens cell line. UAP56 upregulated the protein expression of HSP25 and alpha B-crystallin without impacting their total mRNA levels. Collectively, we hypothesize that HSF4 might recruit UAP56 to couple the downstream transcription and pre-mRNA processing together.

Materials and methods

Cell lines and plasmids

mLEC/hsf4−/− and mLEC/HA-Hsf4 cells were generated in our lab (Zhang et al. 2014). HEK293-phoenix cells were bought from Strategene (La Jolla, USA). HLE-B3 cell line was gifted by Dr. Liu (Huazhong University of Science and Technology). The cells were cultured in DMEM media containing 10% FBS, 100 μg/ml streptomycin, and 100 units/ml penicillin. For the recombinant plasmids pWZL/HA-Hsf4b, human Hsf4b cDNA with HA-tag at the N-terminus was subcloned into the pWZL-Blasticidin vector at the EcoRI restriction site; pbabe-HA-UAP56, the mouse UAP65 cDNA with HA-tag at the N-terminus was cloned into the pBabe-puromycin vector between the BamHI and EcoRI restriction sites; pcDNA-T7-UAP56, the cDNA of UAP56 with T7-tag at N-terminus was subcloned into the PcDNA3 vector at restriction enzymes of BamHI and EcoRI; and pLTHR-shRNA-UAP56, shRNAs targeting UAP56 at sequences of GCCTGAACCTCAAACACAT and GGATTCTTGTGGCTACCAA were cloned into the retroviral vector pLTHR between the BamHI and SalI restriction sites. All of the constructs were confirmed by DNA sequencing.

To establish the stable cell lines, the plasmids pBabe-HA-UAP56, pBabe-puro, pLTHR-shRNA-uap56, and pLTHR-scramble were transiently transfected individually into 239-phoenix cells for 36 h. The cell supernatant containing virus was collected. To make the stable cell lines mLEC/Hsf4−/−, mLEC/+HA-Hsf4, mLEC/Hsf4−/−/HA-UAP56, and mLEC/+HA-HSF4/UAP56, the recombinant retrovirus containing HA-UAP56 or empty vector were mixed with 2 μg/ml of polybrane to infect the mLEC/hsf4−/− and mLEC/+HSF4 cells. To make the mLEC/vector and mLEC/HA-UAP56 stable cell lines, the recombinant retrovirus expressing HA-UAP56 or empty vector were incubated with mLEC/wt cells. The cells were selected in media containing 2 μg puromycin and pooled for future analysis. To make the mLEC/shRNA-uap56 and mLEC/scramble shRNA cell lines, the virus containing shRNA against mouse UAP56 or scramble shRNA were incubated with mLEC/HA-Hsf4 cells. The cells were pooled after being selected in the media containing 2 μg/ml puromycin.

Yeast two hybrid

Matchmaker yeast two hybrid was utilized according to the protocol provided by Clontech to study the proteins that interact with Hsf4b. Briefly, Hsf4b cDNA was subcloned into the bait vector PGBKT7 at the EcoRI restriction site, generating the recombinant plasmid pGBKT7-Hsf4b, that expresses the HSF4b bait protein fused to the C-terminus of the Gal4/DNA-binding domain (Gal4 DNA-BD). PGBKT7-Hsf4b was transiently cotransfected together with the mouse embryonic cDNA library in the pACT2 vector into competent Y2H gold yeast cells. The yeasts that ectopically express pGBKT7-P53 and pACT-SV4-Tag were used as positive controls, while yeasts with the pGBKT7-Hsf4b and pACT empty vectors were used as negative control. The recombinant yeasts were plated on SD/Arg/−trp/−leu/x-gal agar plates and incubated at 25 °C for 3–5 days. The blue yeast colonies on the plates were selected, and the plasmids were extracted and amplified in competent bacteria. The candidate plasmids were verified by DNA sequencing.

Immunoblotting, immunoprecipitation, and GST- pull down assay

The respective protocols for these experiments have been described in our previous papers (Hu et al. 2013).

Semi-quantitative RT-PCR

Total RNA was extracted with Trizol buffer following the accompanying protocol. One microgram of total RNA was used to synthesize the first strand of cDNA with the reverse transcription kit (Promage, USA). The primers used to amplify mouse Hsp25 and αB-crystallin are the following: forward: 5′-caggacgaacatggctaca-3′ and reverse: 5′-agagcgcacagattgacag-3′ (for Hsp25) and forward: 5′-aagaacgccaggacgaacat-3′ and reverse: 5′-gagaggatccacatcggctg-3′ (for αB-crystallin). 18S RNA was used as the internal control. For the semi-quantitative PCR, the samples were pre-denatured at 94 °C for 3 min and then subjected to 25 cycles of 94 °C 30 s, 56 °C 30 s, and 72 °C 50 s. The PCR products were separated on an agarose gel.

Protein-RNA interaction assay

Lens epithelial cell lines or P3 lens were lysed in NP-40 lysis buffer containing RNAase inhibitor. Equal amount of protein lysates was incubated with agarose beads conjugated with rabbit anti-UAP56 antibody at 4 °C for 6 h. The beads were washed in lysis buffer for four to five times. The bead-associated mRNA was extracted with Trizol buffer. The RT-PCR was performed with primers that amplify regions of HSP25 or alpha B-crystallin mRNA. The PCR products were separated on an agarose gel.

Immunofluorescence, immunohistochemistry, and luciferase assay

For immunofluorescence, cells were grown on coverslips and fixed in 3.7% paraformaldehyde for 20 min. The cells were washed with PBST buffer and permeabilized with 0.2% NP-40 buffer for 5 min. After being blocked in 5% BSA buffer for 1 h, the cells were incubated with rabbit antibodies against UAP56 or mouse anti-HA antibody for 1 h followed by Alexa Fluor secondary antibody for 1 h. The nucleus was stained with DAPI. The fluorescence signals were captured with the fluorescence microscope Zeiss 540 under the 1000 index. For immunohistochemistry, the stain-decolorize-stain (SDS) (Li et al. 2014) was used to study the localization association of HSF4 and UAP56 in lens. Briefly, a paraffin-embedded section from P14 mouse lens was subjected to immunohistochemistry. The slide was first immunoreacted with rabbit anti-HSF4 antibody and its corresponding secondary antibody. The signal was developed with AEC kit (DAKO, Denmark) and captured with the microscope; after this, the signal was decolorized by incubating with 80% ethanol at room temperature for 30 min followed by microwaving for 15 min. The same histological section was used to localize UAP56 in the way similar to HSF4. For the luciferase assay, the pGL2-Pcryab construct containing the promoter of alpha B-crystallin and pcDNA-beta-Gal were transiently cotransfected together with pWZL-UAP56 or pWZL-Blasticidin empty vector into the mLEC/hsf4−/− and mLEC/HA-Hsf4 cells. The reported values were obtained by dividing the luciferase value by the beta-gal absorbency values. The error bars reflect three independent experiments.

Results

Hsf4b interacts with DEXD/H-box RNA helicase UAP56

In mammalian cells, HSF4 expresses two splice variants HSF4a and HSF4b (Nakai et al. 1997). HSF4b (here named as HSF4) is the predominant variant whose transcriptional activity is essential for lens development. To identify proteins that associate with HSF4, we performed the yeast two hybrid assay to screen for candidate proteins in the mouse embryonic cDNA library that interact with HSF4. One of the positive clones contained a sequence that is homologous to UAP56 (Fig. 1a). UAP56 (also known as HLA-activated protein 1, BAT1) is a DEXD/H-box RNA helicase that is involved in the regulation of RNA splicing and spliced mRNA nuclear export. To confirm the interaction between HSF4 and UAP56 in lens epithelial cell line (HLE-B3), we cotransfected pcDNA-Flag-HSF4 together with pEBG empty vector or pEBG-UAP56 into HLE-B3 cells; the interaction between Flag-HSF4 and GST-UAP56 was tested by the GST-pull down assays. As the results indicated in Fig. 1, the Flag-HSF4 protein interacts with GST-UAP56 (Fig. 1b lane 1), but not with GST protein alone (Fig. 1b lane 2). Conversely, the ectopic protein of T7-UAP56 was co-precipitated with GST-Hsf4b (196–493) (Fig. 1c lane 3) and weakly with GST-HSF4 (320–493) (lane 4), but not with GST alone and GST-HSF4 (1–230) (Fig. 1c lanes 1 and 2). Furthermore, we found that the amino acids from 230 to 320 in HSF4 are response to interacting with UAP56 (Fig. 1d lanes 2 and 3). These results suggested that UAP56 is a novel HSF4b interacting protein.

HSF4 co-localizes with UAP56 in the nucleus

UAP56 forms the spindle structure in cell nucleus. To determine whether UAP56 and HSF4 are co-localized in nucleus, the construct of pDs-Red-UAP56 was transiently transfected into mLEC/HA-HSF4 cells. The fluorescent results showed that the Ds-Red-UAP56 protein, which is mainly in the nucleus, could co-localize together with HA-HSF4 in the nucleus of mLEC/HA-HSF4 cells (Fig. 2a). Moreover, the nuclear co-localization between the endogenous UAP56 and HA-HSF4 proteins in the mLEC/HA-HSF4 cells was also observed (Fig. 2b). These results suggested that HSF4 is able to associates with UAP56 in the nucleus.

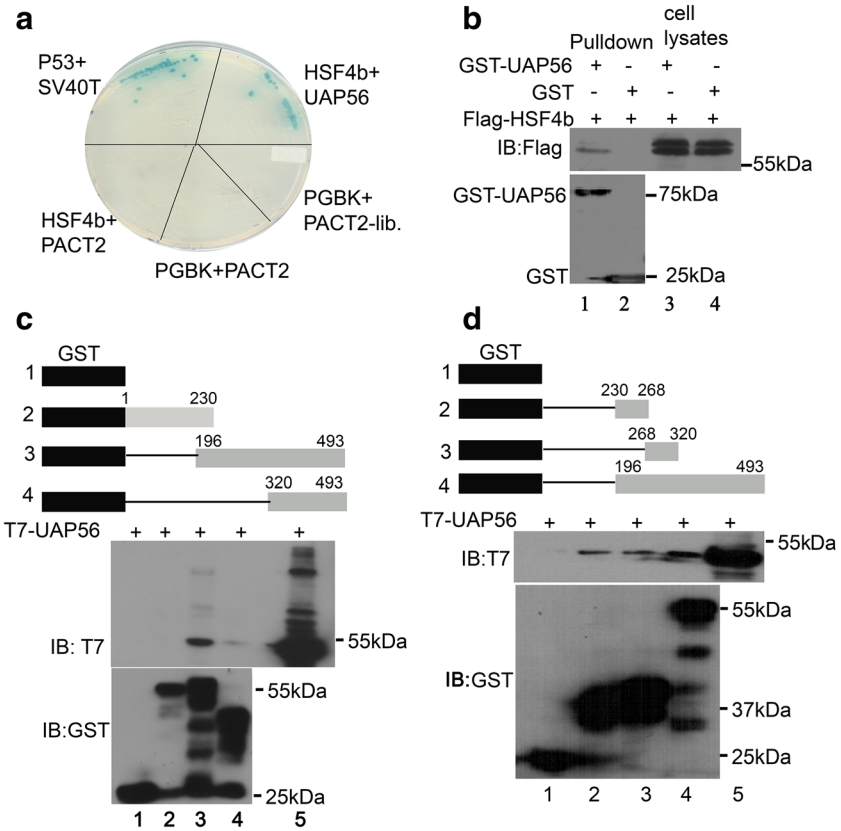

Fig. 1 Hsf4b interacts with UAP56. **a** Matchmaker yeast two hybrid. The tested group, pGBKT7-Hsf4b+pACT-UAP56; positive control, p53 + SV40/Tag; and negative control, PGBKT7-Hsf4b+pACT2 empty vector and pGBKT-7+pACT-cDNA library. **b** GST-pull down assay in vivo. Ectopic Flag-HSF4 protein was pull down with GST-UAP56 (lane 1) or GST alone (lane 2); lanes 3 and 4, immunoblotting Flag-HSF4 protein in the cell lysates using for pull down. **c** To determine the amino acids 196–493 of HSF4 interacts with the T7-UAP56 protein in GST-pull down assay. The ectopic T7-UAP56 protein was pull down with GST alone (lane 1), GST-HSF4 (1–230) (lane 2), GST-HSF4 (196–493) (lane 3), or GST-HSF4 (320–493) (lane 4). The expression of T7-UAP56 in the cell lysates is indicated in lane 5. **d** Using GST-pull down assay to determine the amino acids 230–320 of HSF4 interacts with the T7-UAP56 protein in HLE-B3 cells. The ectopic expression of T7-UAP56 was pull down with GST alone (lane 1), GST-HSF4 (230–268) (lane 2), GST-HSF4 (268–320) (lane 3), and GST-HSF4 (196–493). Lane 5 is the input cell lysates

UAP56 upregulates HSF4-controlled protein expression of HSP25 and alpha B-crystallin

One of the HSF4's functions is to control the expression of heat shock proteins (e.g., HSP25 and alpha B-crystallin) in lens epithelial cells. To determine whether UAP56 regulates HSF4's downstream expression, we firstly measured the regulation of UAP56 on the expression of HSPs by stably expressing HA-UAP56 in wild-type mLEC cells (Fig. 3a). The immunoblotting results indicated that ectopic expression of UAP56 could upregulate the protein expression of HSP70 and alpha B-crystallin, but not that of the HSP25 and HSF1 (Fig. 3a; compared lanes 1 to 2). To determine whether the regulatory roles of UAP56 rely on HSF4, we infected the mLEC/Hsf4−/− and mLEC/HA-HSF4 cells with retrovirus-expressing empty vector or HA-UAP56 to establish four stable cell lines: mLEC/Hsf4−/−, mLEC/Hsf4−/−/HA-UAP56, mLEC/HA-HSF4, and mLEC/HA-HSF4/HA-UAP56 (referred to in the "Materials and methods" section). The immunoblotting results indicated that the protein levels of HSP25 and alpha B-crystallin were significantly upregulated in mLEC/HA-HSF4/HA-UAP56 cells comparing to their expression in mLEC/HA-Hsf4 cells (Fig. 3b lanes 2 and 4), but no difference of their expression levels was observed between mLEC/Hsf4−/−/HA-UAP56 and mLEC/Hsf4−/− cells (Fig. 3b lanes 1 and 3). Interestingly, UAP56 could upregulate HSP70 expression (Fig. 3b lane 3), and that upregulation is inhibited by HSF4 (Fig. 3b lane 4). Knocking down UAP56 by shRNA suppressed HSP25 and alpha B-crystallin expression in mLEC/HA-Hsf4 cells (Fig. 3C; comparing lane 1 to lanes 2 and 3). The results of quantitative RT-PCR indicate that the mRNA levels of HSP25 and alpha B-crystallin in mLEC/HA-HSF4 cells is similar in mLEC/HA-HSF4/HA-UAP56 cells (Fig. 3d bars 2 and 4, bars 6 and 8). No regulatory effect of UAP56 itself on HSP25 and alpha B-crystallin mRNA levels was observed (Fig. 3d bars 3 and 7). Moreover, the luciferase reporter results indicated that UAP56 could not increase HSF4-mediated alpha B-crystallin promoter's

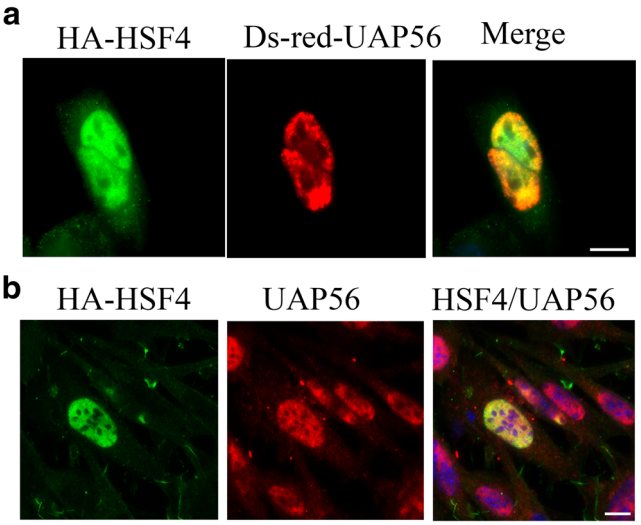

Fig. 2 Co-localization of HSF4 and UAP56 in the nucleus of lens epithelial cells in vitro. **a** The co-localization of Ds-Red-UAP56 and HA-HSF4 in mLEC/HA-HSF4 cells in the immunofluorescent assay. The mLEC/HA-HSF4 cells were transiently transfected with pDs-Red empty vector or pDs-Rad-UAP56. The HA-HSF4 protein was immunoreactive with mouse anti-HA antibody followed by Alexa Fluo 488 goat anti-mouse secondary antibody. Scale bar equals 10 μm. **b** The co-localization of endogenous UAP56 protein with HA-HSF4 in the mLEC/HSF4 cells. The mLEC/HA-HSF4 cells were doubly immunoreacted with mouse anti-HA antibody and rabbit anti-UAP56 antibody, and followed by incubating with the Alexa Fluor 488 goat anti-mouse secondary antibody and Alexa Fluor 594 goat anti-rabbit antibody. The nuclear DNA was stained with DAPI. Scale bar equals 20 μm

activity (Fig. 3e). These results suggest that the UAP56 upregulates the HSP25 and alpha B-crystallin protein expression other than their mRNA expression in HSF4-overexpressed cell lines. UAP56 is involved in regulating pre-mRNA splicing and mature mRNA nuclear exportation. To test whether UAP56 has the regulation on the nuclear exportation of HSP25 and alpha B-crystallin mRNA, we measured the mRNA levels of HSP25 and alpha B-crystallin in the cytosolic and nuclear compartments of the four stable cell lines used in Fig. 3b with the quantitation RT-PCR. The mRNA level of HSP25 and alpha B-crystallin in the cytosolic compartment of the mLEC/HA-Hsf4/HA-UAP56 cells are more than that in the corresponding nuclear compartment (Fig. 4a, b; bars 4 and 8). This phenomenon was not observed in the other three cell lines (Fig. 4a bars 1–3 and 5–7). Using protein-RNA interaction assay, we found that UAP56 can bind to the mRNA of HSP25 or alpha B-crystallin in mLEC/HA-HSF4 cells (Fig. 4c lane 3) and in P3 lens tissues (Fig. 4d lanes 3 and 4). These results imply that UAP56 is involved in regulating the nuclear exportation of HSP25 and alpha B-crystallin mRNA. Collectively, these results revealed that UAP56 upregulates the protein expression of HSP25 and alpha B-crystallin by modulating the latters' mRNA maturation and nuclear exportation.

Both UAP56 and HSF4 developmentally express in the nucleus of lens fiber cells

HSF4 is predominantly expressed in lens epithelial and cortical fiber cells, modulating the proteostasis of postnatal lens development (Cui et al. 2015). The data described in the previous sections suggest that UAP56 is a new associating partner of HSF4. However, it is unclear whether UAP56 and HSF4 associate with each other in the lens. We measured the expression of UAP56 and HSF4 proteins in lens tissue at different ages with immunoblotting, and found that UAP56 and HSF4 were predominantly expressed in P3 and P14 lens, and its expression was hard to be detected in 1- and 2-month lens (Fig. 5a). The immunohistochemistry results suggested that UAP56 expresses predominantly in the nucleus of cortical fiber cells and weakly in the cytoplasm, and its protein density in P14 lens is much more than that in 2-month lens (Fig. 5b) and is consistent with the immunoblotting results (Fig. 5a). Our previous data showed that HSF4 is predominantly in the nucleus of postnatal lens cortical fibers (Cui et al. 2016). Due to the limitation of fluorescent antibody resource, we could not test the co-localization between UAP56 and HSF4 in lens cortical fiber cells with immunofluorescence assay. Instead, we use the peroxidase-based AEC substrate-chromogen (Dako, Denmark) stain-decolorize-stain method (Li et al. 2014) to detect the localization association of HSF4 and UAP56 in P14 lens. As the results indicated in Fig. 5c, the expression pattern of UAP56 is similar to that of HSF4's. Both of them express in the same cortical fiber nuclei in P14 lens.

Discussion

In this study, we found that HSF4 could interact with UAP56, a factor that plays multiple functions in the regulation of pre-mRNA splicing and mature mRNA nuclear export. Our data indicated that HSF4 and UAP56 co-localized with each other in lens epithelial cells. UAP56 could upregulate HSF4-controlled protein expression of HSP25 and alpha B-crystallin in lens epithelial cells without impacting the latter's total mRNA levels. Moreover, UAP56 could increase the mRNA ratio of HSP25 and alpha B-crystallin in cytosolic verse nuclear (Fig. 3d). These data implied that UAP56 might function as a downstream factor to modulate the downstream transcription processing of HSF4 (such as pre-mRNA splicing and mature mRNA nuclear export), rather than directly regulating HSF4's transcription. The data presented some novel clues of how HSF4 couples the posttranscriptional machine in regulating its downstream mRNA processing.

UAP56 comprises the splicesome complex and TREX complex regulating pre-mRNA splicing and mRNA nuclear export (Dufu et al. 2010; Libri et al. 2001). Pre-mRNA synthesis, splicing, and nuclear export are coupled to each other

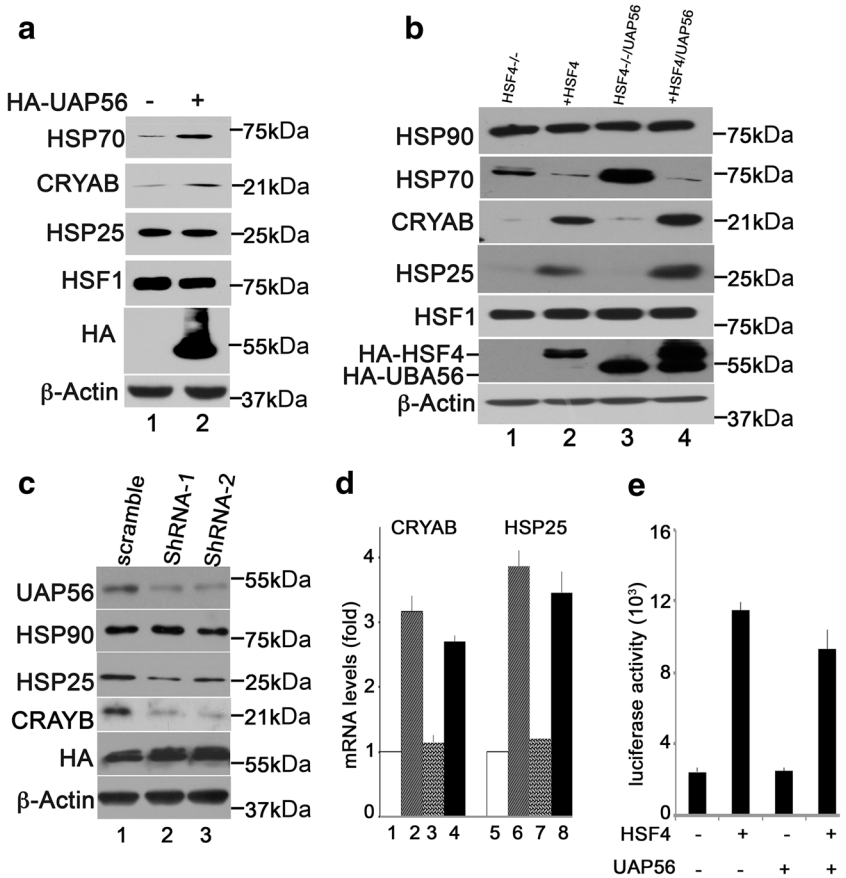

Fig. 3 UAP56 upregulates HSF4-controlled HSP25 and alpha B-crystallin protein expression**. a** UAP56 upregulates the expression of heat shock proteins in wild-type mLEC cells. Stable cell lines mLEC/wt (the wild-type mLEC cells infected with empty recombinant retrovirus, lane 1) and mLEC/HA-UAP56 cells (mLEC/wt cells was infected with the retrovirus expressing HA-UAP56, lane 2) were immunoblotted with antibodies against HSP25, alpha B-crystallin (CRYAB), HSP70, HSF1, and beta-Actin. **b** Immunoblotting the expression of HSP90, HSP70, CRYAB, HSP25, HSF1, HA-tag, and beta-Actin in the stable cell lines of mLEC/Hsf4−/− (lane 1), mLEC/HA-Hsf4 (lane 2, HA-HSF4 was reconstituted into mLEC/Hsf4−/− cells), mLEC/Hsf4−/−/HA-UAP56 (UAP56 was recapitulated into mLEC/Hsf4−/− cells, lane 3), and mLEC/HA-HSF4/HA-UAP56 (UAP56 was reconstituted into the mLEC/HA-HSF4 cells, lane 4). **c** Knocking down UAP56 by shRNA downregulates the protein expression of HSP25 and alpha B-crystallin in mLEC/HA-Hsf4 cells. The mLEC/HA-HSF4 cells were infected with retrovirus expressing the scramble shRNA (lane 1), shRNA against UAP56/no. 1 (shRNA-1, lane 2) and shNRA/no. 2 (shRNA-2, lane 3). The protein expression of HSP25, CRYAB, HSP90, UAP56, HA-HSF4, and beta-Actin were immunoblotted with their antibodies. **d** Quantitative RT-PCR to determine the mRNA expression of HSP25 and alpha B-crystallin in the cells used in **b**. **e** Luciferase reporter assay, the constructs of pGL-PCrayb (the promoter fragment of Cryab was subcloned to the uprestream of luciferase in the pGL-2 basic vector) and pcDNA-beta-Gal (used for internal control) were transiently transfected into mLEC/HSF4−/− (Hsf4−/−) together with sham vector (bar 1), HSF4 (bar 2), UAP56 (bar 3), or HSF4 plus UAP56 (bar 4). The luciferase and beta-galactosidase were measured following the protocol provided by the kits. The bar represents standard deviation of three independent experiments

and regulate each other through protein-protein associations (Listerman et al. 2006). For example, the association between SR factors and RNAPII couples pre-mRNA synthesis with RNA splicing (Ji et al. 2013), and the interaction between the splicing factor snRNP and transcription elongation factor reflects the regulation of splicing on RNA synthesis (Fong and Zhou 2001). Our data suggested that HSF4 could recruit UAP56 to regulate downstream mRNA processing. The supporting evidences are the following: (1) UAP56 upregulates the HSF4-controlled protein expression of HSP25 and alpha B-crystallin without impacting on the latter's mRNA level; (2) knocking down UAP56 suppresses HSP25 protein expression; (3) UAP56 interacts with HSP25 and alpha B-crystallin mRNA; and (4) by coupling with HSF4, UAP56 increases cytosolic mRNA levels of HSP25 and alpha B-crystallin compared to the nuclear fraction. According to these data, we hypothesize that HSF4 may recruit UAP56 to regulate HSP25 and alpha B-crystallin's mRNA nuclear export. However, whether UAP56 is involved in regulating HSP25 and alpha B-crystallin, pre-mRNA splicing is still under investigation.

UAP56 upregulated HSP70 expression when it was ectopically expressed in mLEC/Wt cells (Fig. 3a). Moreover, the protein levels of HSP70 were significantly upregulated in mLEC/Hsf4−/−/UAP56 cells comparing to its levels in mLEC/Hsf4−/− cells (Fig. 3b lanes 1 and 3). This suggests

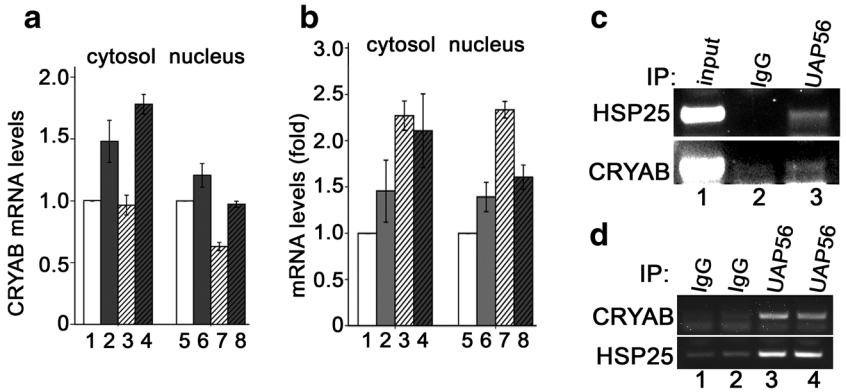

Fig. 4 UAP56 interacts with and promotes cytosolic mRNA of HSP25 and alpha B-crystallin. **a** and **b** Quantitative RT-PCR to determine the mRNA expression of alpha B-crystallin (**a**) and HSP25 (**b**) in the cytoplasm (bars 1–4) and nuclear (bars 5–8) compartments in the cells used in Fig. 3B. **c** UAP56 interacts with HSP25 and CRYAB mRNA in mLEC/HA-HSF4 cells. The mLEC/HA-HSF4 cells were used to immunoprecipitate by antibodies against UAP56 (lane 3) or sham IgG (lane 2). The mRNAs of HSP25 and CRYAB that are co-immunoprecipitated with UAP56 were measured with RT-PCR. Lane 1, imputed total mRNA. **d** UAP56 interacts with HSP25 and alpha B-crystallin mRNAs in P3 mouse lens. Lanes 1 and 2 are sham IgG; lanes 3 and 4 represent the samples of four lenses of P3 mice tested with antibody against UAP56

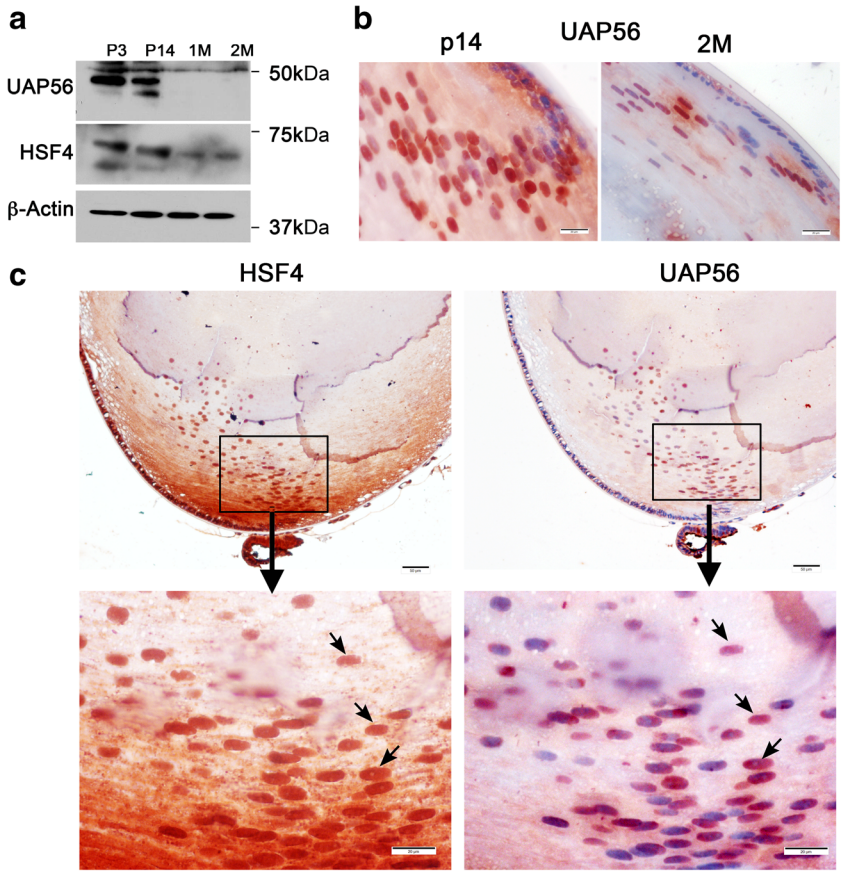

Fig. 5 Expression of UAP56 and HSF4 in lens tissues. **a** Immunoblotting the expression of UAP56 and HSF4 in lens tissues. Mouse lenses were collected at age of P3 (lane 1), P14 (lane 2), P28 (lane 3), and 2M (lane 4) and subjected to immunoblotting with antibodies against UAP56, HSF4, and beta-Actin. **b** The expression of UAP56 in the lens of P14 and 2-month-old mice in immunohistochemistry assay. The cell nucleus was stained with hematoxylin. **c** The localization of UAP56 and HSF4 proteins in P14 lens. One paraffin-embedded section was incubated with rabbit antibodies against HSF4 followed by incubation with HRP-conjugated goat anti-rabbit secondary antibodies. The immunoreaction was visualized using the peroxidase-based AEC substrate-chromogen (Dako, Denmark) and photographed under the microscope. After this, the antibodies to HSF4 were removed. The tissue section was decolorized and subjected to immunohistochemistry with rabbit antibody against UAP56 and HRP-conjugated goat anti-rabbit secondary antibodies. The immunoreaction was again visualized using the peroxidase-based AEC substrate-chromogen and photographed. The arrows indicate HSF4 and UAP56 in the same fiber cell nuclei. Boxes in low-magnification images indicate position of the high-magnification images. The scale bars represent 50 μm in low-magnification images and 20 μm in high-magnification images

that in addition to HSP25 and alpha B-crystallin, UAP56 is also involved in regulating HSP70 expression, and its upregulation is independent of HSF4 (Fig. 3). HSF4 suppressed UAP56-mediated HSP70 protein expression (Fig. 3b lanes 1 and 3). Our previous report demonstrated that HSF4 could inhibit HSP70 expression by interacting with and destabilization of HSF1. According to the results in Fig. 3, we proposed that UAP56 might couple HSF1 to increase HSP70 mRNA nucleus exportation and HSP70 translational efficiency. HSF4 suppresses UAP56-mediated HSP70 protein expression by either directly suppressing HSP70 mRNA transcription or by selectively inhibiting UAP56's mRNA nuclear exportation, and this hypothesis is still under the investigation in our lab.

Conclusion

In this study, we present a potential regulatory coordination between HSF4-mediated transcription and posttranscriptional processing in the regulation of heat shock protein expression. The interaction between HSF4 and UAP56 might enhance the downstream mRNA maturation and nuclear exportation, increasing the translational efficiency. This presents new clues for uncovering the association of HSFs' transcription to the mRNA processing.

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