HSF-1–mediated cytoskeletal integrity determines thermotolerance and life span

Nathan A. Baird *et al.*

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AGING

HSF-1–mediated cytoskeletal integrity determines thermotolerance and life span

Nathan A. Baird,[1]* Peter M. Douglas,[1]* Milos S. Simic,[1] Ana R. Grant,[2] James J. Moresco,[3] Suzanne C. Wolff,[1] John R. Yates III,[3] Gerard Manning,[4] Andrew Dillin[1]†

The conserved heat shock transcription factor–1 (HSF-1) is essential to cellular stress resistance and life-span determination. The canonical function of HSF-1 is to regulate a network of genes encoding molecular chaperones that protect proteins from damage caused by extrinsic environmental stress or intrinsic age-related deterioration. In *Caenorhabditis elegans*, we engineered a modified HSF-1 strain that increased stress resistance and longevity without enhanced chaperone induction. This health assurance acted through the regulation of the calcium-binding protein PAT-10. Loss of *pat-10* caused a collapse of the actin cytoskeleton, stress resistance, and life span. Furthermore, overexpression of *pat-10* increased actin filament stability, thermotolerance, and longevity, indicating that in addition to chaperone regulation, HSF-1 has a prominent role in cytoskeletal integrity, ensuring cellular function during stress and aging.

The survival of an organism is intricately linked to its ability to maintain cellular quality control, including organelle integrity, lipid homeostasis, proper protein folding, and cellular communication. The organismal response to unpredictable environmental changes is critical to mitigate damages caused by stress. Genes encoding the heat shock protein (HSP) family of molecular chaperones show the largest transcriptional increase in response to thermal stress, suggesting that these proteins are part of a fundamental defense against proteotoxic stress. Consistent with this hypothesis, ectopic expression of the master transcriptional regulator of HSPs, HSF-1, is sufficient to confer resistance to thermal stress and increase life span in the nematode *Caenorhabditis elegans* (*1*). Furthermore, *hsf-1* overexpression can alleviate toxicity associated with diseases caused by misfolded or aggregated proteins (*2*).

However, chaperones may be dispensable for thermotolerance and longevity. Neither a hypomorphic mutation of *hsf-1*, nor preventing the up-regulation of HSPs affects thermotolerance of *C. elegans* (*3*, *4*). However, other studies using the same *hsf-1* mutant show decreased heat resistance (*5*). The conflicting data may result from differences in experimental design, but it is clear that HSF-1 function is not fully explained by chaperones mediating stress resistance and life-span determination.

To test for protective mechanisms independent of enhancing chaperone induction, we generated

[1]Howard Hughes Medical Institute, University of California Berkeley, Berkeley, CA 94720, USA. [2]Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI 48109, USA. [3]Scripps Research Institute, La Jolla, CA 92037, USA. [4]Genentech, South San Francisco, CA 94080, USA. *These authors contributed equally to this work. †Corresponding author. E-mail: dillin@berkeley.edu

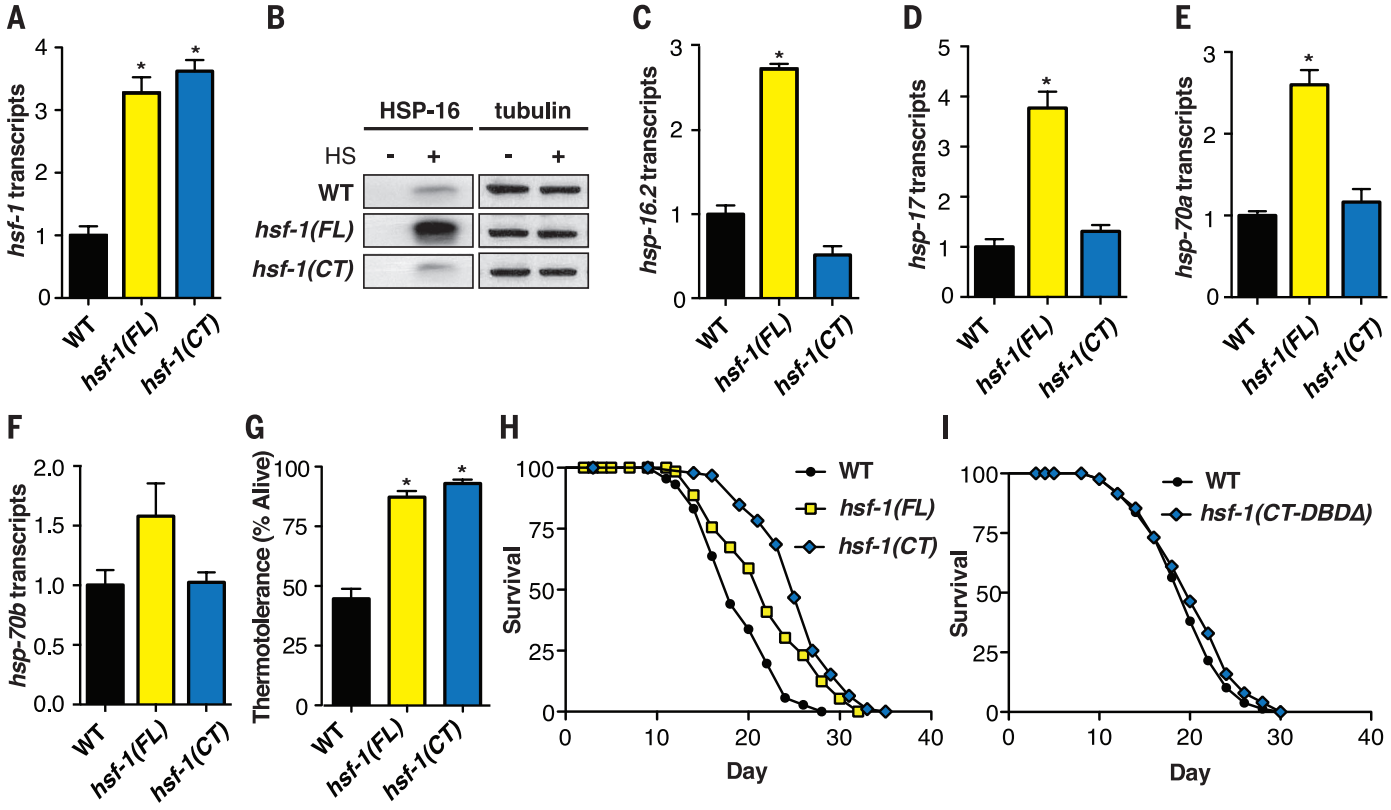

Fig. 1. *hsf-1(CT)* increases life span and thermotolerance without enhancing chaperone induction. (**A**) Equal overexpression of *hsf-1(FL)* and *hsf-1(CT)* determined by means of quantitative polymerase chain reaction (PCR). (**B**) Western blot of HSP-16 before and after heat shock (HS). (**C** to **F**) Quantitative PCR of (C) *hsp-16.2*, (D) *hsp-17*, (E) *hsp-70a* (C12C8.1), and (F) *hsp-70b* (F44E5.4) show enhanced induction in *hsf-1(FL)*. (**G**) Thermotolerance assay of WT, *hsf-1(FL)*, and *hsf-1(CT)* worms shifted from 20° to 34° for 13 hours. (**H**) Life-span survival curves of WT, *hsf-1(FL)*, and *hsf-1(CT)* strains. (**I**) Life-span survival curves of WT and *hsf-1(CT)* strains with the DNA-binding domain deleted (*CT-DBDΔ*). *$P < 0.005$; error bars indicate SEM.

transgenic nematodes overexpressing full-length *hsf-1* [*hsf-1(FL)*] or a *hsf-1* C-terminal truncation [*hsf-1(CT)*]. The *hsf-1(CT)* variant was designed to mimic the C-terminal missense mutation found in the *hsf-1(sy441)* mutant, a widely used allele that decreases stress-induced HSP transcription via the removal of a transactivation domain (*6*). *hsf-1(FL)* was overexpressed in the N2 wild-type (WT) background, and *hsf-1(CT)* was overexpressed in the *hsf-1(sy441)* mutant. Therefore, the *hsf-1(CT)* strain mirrored the overexpression of *hsf-1(FL)* but contained no endogenous copies of full-length *hsf-1* (fig. S1). Both transgenes were expressed threefold higher than endogenous *hsf-1* (Fig. 1A).

Analysis of protein and transcript abundance confirmed that overexpression of *hsf-1(FL)* enhanced heat-inducible expression of all HSPs tested, whereas *hsf-1(CT)* caused no difference from wild type (Fig. 1, B to F, and fig. S2). Yet, both *hsf-1(FL)* and *hsf-1(CT)* transgenic worms had increased thermotolerance and life span (Fig. 1, G and H). The life-span extension of *hsf-1(CT)* was unexpected, so we tested whether this phenotype was dependent on a functional DNA-binding domain. Increased longevity was abolished upon removal of the DNA binding domain (*hsf-1(CT-DBDΔ)*) (Fig. 1I). Thus, increased life span and thermotolerance did not correlate with enhanced HSP transcription.

To find other cellular networks that contribute to HSF-1–mediated stress resistance and longevity assurance, we performed quantitative transcriptomic and proteomic analyses comparing *hsf-1(FL)* and *hsf-1(CT)* strains with WT and *hsf-1(sy441)* strains. We filtered for transcripts or proteins that showed increased abundance, under basal or heat-stressed conditions, exclusively in the heat-protected strains (fig. S3). The 98 genes that met our filtering criteria were enriched for functions in development, cytoskeleton organization, complex assembly, and immune defense response (fig. S4).

Reduced expression of genes essential to thermotolerance should lower survival under heat stress. Therefore, we performed a RNA interference (RNAi)–based thermotolerance screen on the genes that passed our filtering criteria (fig. S5 and table S1). From the screen, we identified a troponin-like calcium-binding protein, PAT-10, as essential for thermotolerance (Fig. 2A). Transcription of *pat-10* was heat-inducible in all strains (Fig. 2B). Furthermore, *hsf-1* overexpression strains showed an increase in *pat-10* transcripts under basal and heat-stress conditions (Fig. 2B). After examining the upstream promoter region of *pat-10*, a putative binding site for HSF-1 (*7*, *8*) was identified within 500 base pairs of the transcription start site (fig. S6). Additionally, *hsf-1* RNAi blocked the up-regulation of *pat-10* upon heat shock (Fig. 2C). Therefore, *pat-10* appears to be a direct target of HSF-1 transcriptional regulation.

Because loss of *pat-10* expression reduced thermotolerance, we tested whether ectopic overexpression of *pat-10* could render animals more thermotolerant. Twofold overexpression of *pat-10* (Fig. 2D) significantly increased heat protection (Fig. 2E) and extended life span (Fig. 2F). Furthermore, RNAi of *pat-10* eliminated the increased thermotolerance (Fig. 2E) and life span (Fig. 2F) of the *pat-10* overexpression strain. *pat-10* RNAi also abolished the extended life spans of the *hsf-1* overexpression strains (Fig. 2G). Thus, *pat-10* appears to be necessary and sufficient for increased thermotolerance and longevity. Additionally, the beneficial effects of *pat-10* overexpression were not due to increases in basal HSP transcription (fig. S7). One function of *pat-10* is its role in the troponin complex (*9*–*11*), which is necessary for the contraction of body wall muscles.

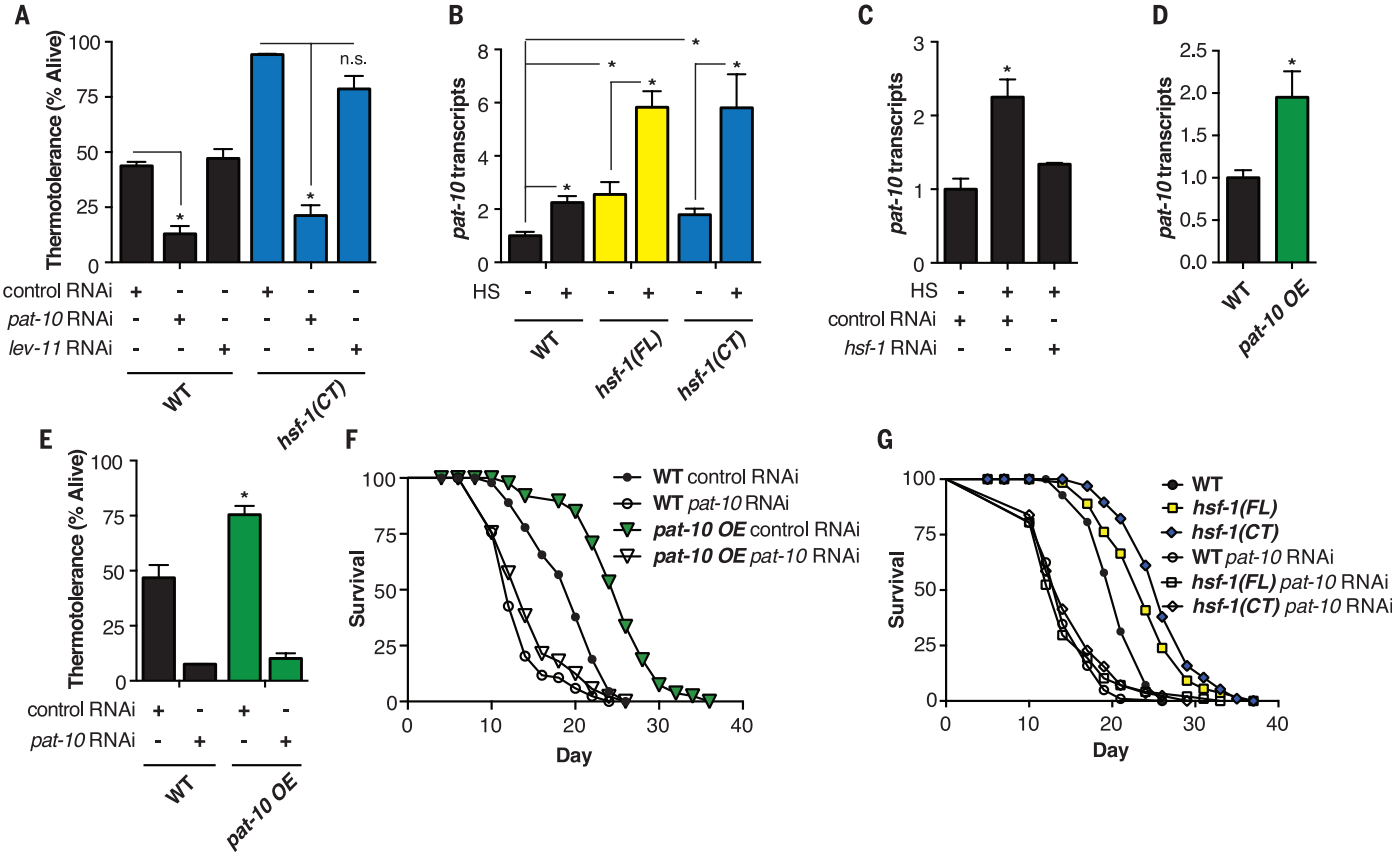

Fig. 2. *pat-10* is necessary and sufficient for thermotolerance and longevity. (**A**) Thermotolerance of WT and *hsf-1(CT)* strains treated with *pat-10* or *lev-11* RNAi. (**B**) Quantitative PCR of *pat-10* with and without heat shock (HS). (**C**) Effect of *hsf-1* RNAi on *pat-10* transcription upon heat shock. (**D**) Quantified expression of *pat-10* in the *pat-10 OE* strain. (**E**) Effect of *pat-10* overexpression or *pat-10* RNAi on thermotolerance. (**F**) Life-span survival curves for WT or *pat-10*–overexpressing strains treated with control or *pat-10* RNAi. (**G**) Life-span survival curves for WT or *hsf-1*–overexpressing strains treated with control or *pat-10* RNAi. *$P < 0.05$; error bars indicate SEM.

However, RNAi toward the worm homolog of tropomyosin—*lev-11*, a partner with *pat-10* in the troponin complex—did not affect heat resistance (Fig. 2A). This suggests that the role of *pat-10* in muscle contraction does not influence thermotolerance.

Loss of *pat-10* also disrupts actin cytoskeleton dynamics and endocytosis (*10*–*13*). To address these potential mechanisms of protection, we used green fluorescent protein (GFP)–labeled muscle filaments to assess actin organization (*14*). Upon heat shock, muscle filaments became unorganized and damaged, leading to impaired motility (Fig. 3, A and B). However, *pat-10* overexpression was sufficient to prevent heat-induced muscle and motility deterioration (Fig. 3, A and B). Furthermore, heat shock decreased the ratio of the filamentous (F) actin to globular (G) actin in WT worms, whereas the protected *pat-10* overexpression animals maintained F actin upon exposure to heat stress (Fig. 3C and fig. S8). With regard to aging, the ratio of F to G actin also decreased with age, and *pat-10* overexpression lessened this decline (Fig. 3D and fig. S8). Hence, under conditions of acute stress or gradual age-related deterioration, the integrity of the actin cytoskeleton is correlated with organismal survival, and overexpression of *pat-10* can abrogate the collapse of actin filaments.

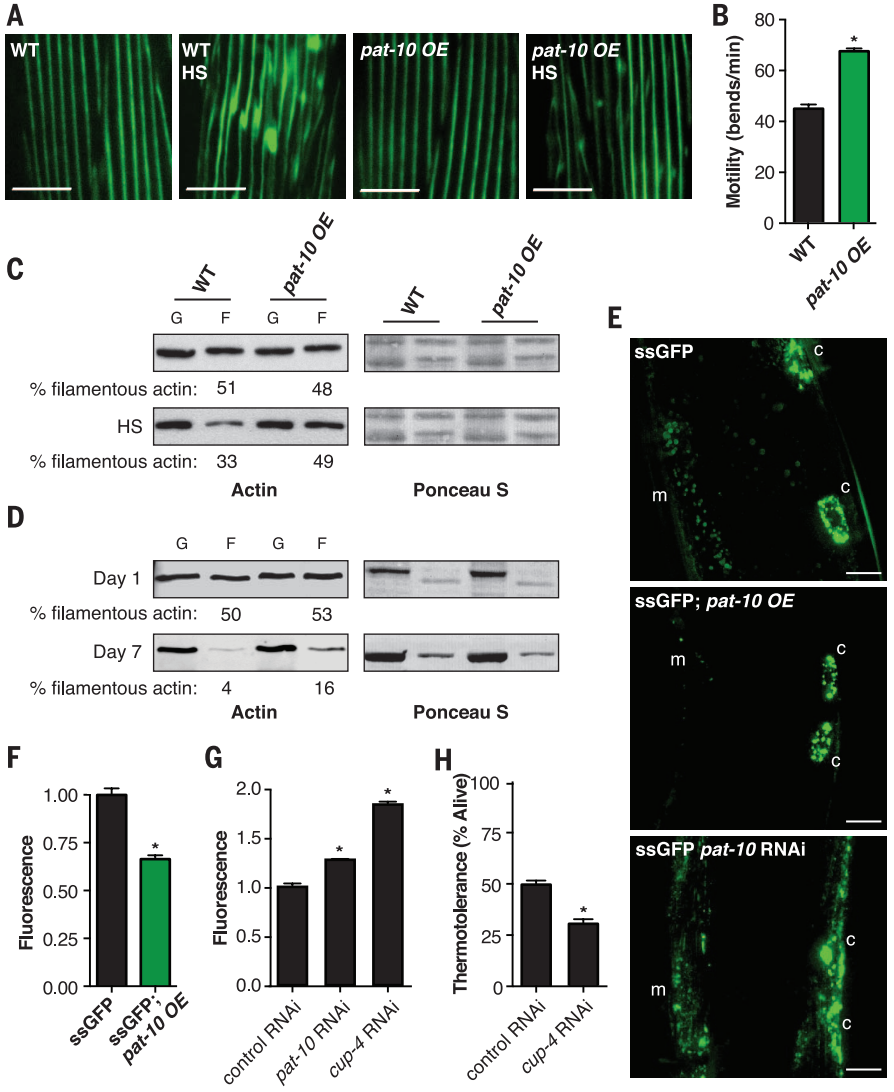

Fig. 3. *pat-10* overexpression improves actin cytoskeletal integrity and cellular trafficking. (**A**) GFP-tagged myosin heavy chain in muscle detected by means of fluorescent microscopy, before and after heat shock (HS). (**B**) Worm thrashes per minute in liquid to monitor motility after heat shock. (**C** and **D**) Abundance of filamentous (F) actin or globular (G) actin after (C) heat shock or (D) aging. Ponceau S staining shown as a loading control. (**E**) Microscopy showing ssGFP derived from muscle cells (m), endocytosed by coelomocytes (c) to be degraded. (**F**) Normalized ssGFP fluorescence quantification with and without *pat-10* overexpression. (**G**) Effect of blocking coelomocytic endocytosis on GFP fluorescence in the ssGFP reporter strain. (**H**) Thermotolerance after RNAi of *cup-4*. *$P < 0.05$; error bars indicate SEM. Scale bars, 10 μm.

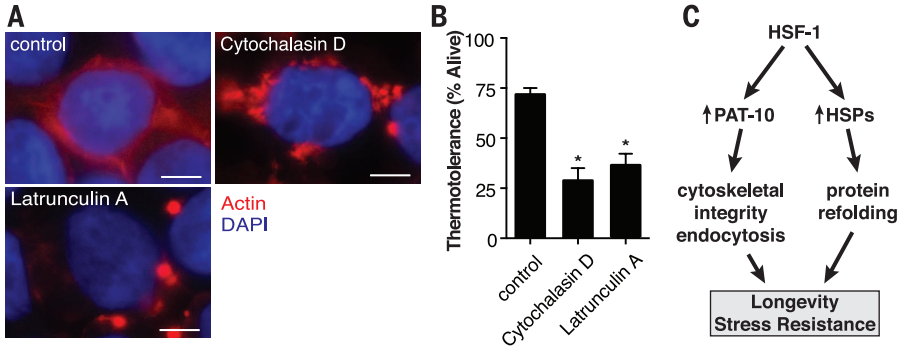

Fig. 4. Impairing actin dynamics decreases thermotolerance in mammalian cell culture. (**A**) Microscopy of HEK293T cells treated with cytochalasin D or latrunculin A [phalloidin stain of actin in red, 4′,6-diamidino-2-phenylindole (DAPI) stain of DNA in blue]. (**B**) Thermotolerance of HEK293T cells after a heat shock at 45°C for 2 hours treated with cytochalasin D or latrunculin A. (**C**) Proposed model of the dual pathways of HSF-1–mediated health assurance. *$P < 0.05$; error bars indicate SEM. Scale bars, 5 μm.

RNAi-induced loss of *pat-10* disrupts endocytosis through impairment of the actin cytoskeleton (*12*, *13*, *15*). To assay the role of *pat-10* in endocytosis, we used a secretion and endocytosis reporter designed to actively secrete GFP (ssGFP) from muscle cells into the pseudocoelomic fluid, where it is endocytosed by the coelomocyte cells and degraded (fig. S9A) (*16*). Therefore, the ssGFP reports upon effective muscular secretion and endocytosis by coelomocytes. Fitting the hypothesis that *pat-10* overexpression improves transport and cellular processing through improved subcellular scaffolding, the *pat-10 OE* strain had a decrease in overall ssGFP fluorescence (Fig. 3, E and F). The decrease in ssGFP resulted from improved secretion and uptake, as shown by the absence of fluorescence in the muscle and pseudocoelomic fluid (Fig. 3E). This decrease was not due to an overall decrease in expression of GFP (fig. S9B). Conversely, RNAi of *pat-10* increased overall fluorescence through decreased muscle secretion and coelomocytic endocytosis (Fig. 3, E and G). To fully block coelomocytic uptake and degradation of ssGFP, RNAi of *cup-4*, a ligand-gated ion channel required in endocytosis (*17*), showed an even higher increase in fluorescence (Fig. 3G) and also reduced thermotolerance in the wild type (Fig. 3H). Collectively, these data indicate *pat-10* has an active role in cytoskeletal maintenance, which is critical to cellular transport.

To test for conservation, we disrupted the actin cytoskeleton in human embryonic kidney (HEK) 293T cells using cytochalasin D, which blocks the addition of actin monomers to filaments (*18*), or latrunculin A, which binds actin monomers and prevents polymerization (Fig. 4A) (*19*). Inhibiting filamentous actin formation with either cytochalasin D or latrunculin A significantly reduced thermotolerance in human cells without causing death at permissive temperatures (Fig. 4B and fig. S10). Similar to our *C. elegans* data, these findings reiterate the importance of the actin cytoskeleton during times of cellular stress.

Elevated levels of *hsf-1* have been shown to benefit multiple organisms, yet its oncogenic properties are a major therapeutic drawback (*20*, *21*). Because the inducible chaperone network promotes survival and proliferation of metastasizing cells (*22*), the ability to harness protective, non-chaperone components within the HSF-1 signal transduction cascade appears essential for future drug development. Identification of *pat-10* as a modifier of thermotolerance and longevity may apply to mammalian systems without the typical oncogenic dangers associated with increased chaperone levels.

The *hsf-1(CT)* strain was still able to mount a transcriptional response to heat shock, albeit reduced in complexity of *hsf-1(FL)*. The molecular mechanism remains unclear by which *hsf-1(CT)* regulates transcription without the C-terminal activation domain, but possible explanations include HSF-1 containing multiple activation domains. Alternatively, the *hsf-1(CT)* modification may alter affinities to DNA-binding sites or different cofactors, which would modify the transcriptional profile.

Our findings underscore the importance of maintaining filamentous actin, as opposed to total levels of actin. We propose a model in which HSF-1 regulates chaperones and actin cytoskeletal genes in parallel to promote thermotolerance and longevity (Fig. 4C). In the absence of chaperone induction, stabilization of the actin cytoskeleton is sufficient to promote survival under conditions of cellular stress and aging.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6207/360/suppl/DC1
Materials and Methods
Figs. S1 to S10
Tables S1
References (*23*–*40*)

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AUTOIMMUNITY

Detection of T cell responses to a ubiquitous cellular protein in autoimmune disease

Yoshinaga Ito,[1] Motomu Hashimoto,[1,2,3,4]* Keiji Hirota,[2]* Naganari Ohkura,[2,5] Hiromasa Morikawa,[2] Hiroyoshi Nishikawa,[2] Atsushi Tanaka,[2,5] Moritoshi Furu,[3,6] Hiromu Ito,[3,6] Takao Fujii,[3,4] Takashi Nomura,[1] Sayuri Yamazaki,[7] Akimichi Morita,[7] Dario A. A. Vignali,[8,9] John W. Kappler,[10,11] Shuichi Matsuda,[6] Tsuneyo Mimori,[4] Noriko Sakaguchi,[2] Shimon Sakaguchi[1,2,12]†

T cells that mediate autoimmune diseases such as rheumatoid arthritis (RA) are difficult to characterize because they are likely to be deleted or inactivated in the thymus if the self antigens they recognize are ubiquitously expressed. One way to obtain and analyze these autoimmune T cells is to alter T cell receptor (TCR) signaling in developing T cells to change their sensitivity to thymic negative selection, thereby allowing their thymic production. From mice thus engineered to generate T cells mediating autoimmune arthritis, we isolated arthritogenic TCRs and characterized the self antigens they recognized. One of them was the ubiquitously expressed 60S ribosomal protein L23a (RPL23A), with which T cells and autoantibodies from RA patients reacted. This strategy may improve our understanding of the underlying drivers of autoimmunity.

T cells mediate a variety of autoimmune diseases (*1*, *2*), likely through the recognition of self antigens. However, identification of the self antigens targeted by T cells in systemic autoimmune diseases such as rheumatoid arthritis (RA) has been technically difficult (*3*–*5*). This is because pathogenic T cells expressing high-affinity T cell receptors (TCRs) for ubiquitous self antigens may be largely deleted (i.e., negatively selected) in the thymus and