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HSF-1 mediated cytoskeletal integrity determines thermotolerance and lifespan

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Abstract

The conserved transcription factor HSF-1 is essential to cellular stress resistance and organismal lifespan determination. The canonical function of HSF-1 is to regulate a network of molecular chaperones that maintain protein homeostasis during extrinsic environmental stresses or intrinsic age related deterioration. In the metazoan *C. elegans*, we engineered a modified HSF-1 strain that increases stress resistance and longevity without enhancing chaperone induction. This HSF-1 dependent health assurance acts through the regulation of *pat-10*. Upon heat stress *pat-10* upregulation maintains a functional actin cytoskeleton and endocytic network. Loss of *pat-10* causes a collapse of organismal health and failure of stress resistance. Furthermore, overexpression of *pat-10* is sufficient to increase both thermotolerance and longevity by mechanisms that affect actin stability. Our findings indicate that in addition to chaperone induction, HSF-1 plays a prominent role in cytoskeletal integrity to ensure proper cellular function during times of 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 or extreme environmental changes is critical to mitigate damages caused by cellular stress. The heat shock protein (HSP) family of molecular chaperones is the most highly induced class of genes in response to thermal stress, suggesting these proteins are part of a fundamental defense against proteotoxic stress. Consistent with this hypothesis, ectopic expression of the master transcriptional regulator of HSPs, heat shock factor-1 (HSF-1), is sufficient to confer resistance to thermal stress and increase lifespan in the nematode *C. elegans* (*1*). Furthermore, overexpression of HSF-1 can alleviate the toxicity associated with diseases caused by misfolding or aggregating proteins (*2*).

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Interestingly, recent studies have begun to reveal the potential dispensability of chaperone induction in thermotolerance and longevity. For example, neither a hypomorphic mutation of *hsf-1*, nor a block in the transcriptional upregulation of HSPs using α-amanitin affects the thermotolerance of *C. elegans* under conditions of heat stress (*3, 4*). However, other studies using the same mutant strain of *hsf-1* find a significant decrease in heat stress resistance (*5*). The conflicting results may be due to experimental design differences, but taken together suggest a complexity in HSF-1 regulation and function that is not fully captured in traditional chaperone induction models of stress resistance and aging.

To test potential HSF-1 mediated mechanisms of cellular protection that are independent of enhancing chaperone induction, integrated transgenic nematode strains were generated that overexpressed either the full-length *hsf-1* gene (*hsf-1(FL)*) or a variant of *hsf-1* with a C-terminal truncation (*hsf-1(CT)*) of 84 amino acids, removing a transcriptional activation domain responsible for the upregulation of HSPs (*6*). Importantly, the *hsf-1(CT)* variant was designed to mimic the C-terminal missense mutation found in the *hsf-1(sy441)* mutant strain, a widely used allele that decreases stress induced HSP transcription (*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 background. Therefore the *hsf-1(CT)* strain mirrored the overexpression of *hsf-1(FL)* but contained no endogenous copies of full length, wild-type *hsf-1* (Fig. 1A). Both transgenes used the ubiquitous *sur-5* promoter, resulting in approximately 3-fold higher transcriptional expression than endogenous *hsf-1* expression (Fig. 1B).

Analysis of protein and transcript abundance confirmed that overexpression of *hsf-1(FL)* enhanced *hsp-16* heat inducible expression, whereas *hsf-1(CT)* overexpression showed no difference to the wild-type control strain (Fig. 1, C and D). While *hsf-1(CT)* overexpression had no effect, *hsf-1(FL)* worms also showed enhanced transcriptional upregulation of all HSF-1 regulated HSPs tested (Fig. 1, E to G). Furthermore, we performed transcriptome sequencing analysis of these strains and confirmed *hsf-1(FL)* enhanced transcription of all known heat inducible HSPs, whereas *hsf-1(CT)* did not (fig. S1).

Interestingly, both *hsf-1(FL)* and *hsf-1(CT)* transgenic worms had increased thermotolerance (Fig. 1H). Furthermore, both strains lived significantly longer than wild-type (Fig. 1I). Because the lifespan extension of *hsf-1(CT)* was unexpected, we tested if this phenotype was dependent on a functional DNA binding domain. We found that the increased longevity was abolished when the DNA binding domain was removed (*hsf-1(CT-DBDΔ)* (Fig. 1J). Taken together, increased lifespan and thermotolerance did not correlate with the induction of HSPs. These findings support a hypothesis in which thermotolerance and longevity of an organism mediated by overexpression of *hsf-1* is independent of increased induction of chaperones.

Intrigued by the findings that *hsf-1(CT)* can regulate thermotolerance without enhanced HSP induction, we sought to find factors that were responsible for HSF-1 mediated thermotolerance. To determine which cellular networks are required in these long-lived, thermo-protected worms, we completed quantitative transcriptomic and proteomic analyses comparing *hsf-1(FL)* and *hsf-1(CT)* strains to wild-type and *hsf-1(sy441)* strains. We filtered

for significantly upregulated transcripts or proteins, at either basal or heat stress conditions, unique to our thermotolerant strains (Fig. 2A). This filtering method only considered candidates that were similarly upregulated in the *hsf-1(FL)* strain, avoiding potential neomorphic effects of the *hsf-1(CT)* strain.

Fifty-two gene products passed our filters using the proteomic data and 46 genes met these criteria from the transcript data. The corresponding 98 unique genes had significant gene ontology enrichments for development, cytoskeleton organization, complex assembly, and immune defense response (fig. S2). Significantly, molecular chaperones were absent from the enriched gene ontologies.

Reducing expression of genes essential to thermotolerance should lower survival under heat stress. Therefore, we performed an RNAi based screen in the *hsf-1(CT)* strain of the 98 genes that passed our filtering criteria. Eighty-four genes were scored for their effect on thermotolerance (fig. S3). RNAi of the remaining 14 genes induced developmental arrest and were not further characterized. From the screen we identified a calcium binding protein, *pat-10*, as essential for thermotolerance (Fig. 2B).

Significantly, *pat-10* transcription was heat inducible in all strains, including wild-type (Fig. 2C). Furthermore, *hsf-1* overexpression strains showed a basal increase in *pat-10* transcripts, as well as an enhanced induction upon thermal stress (Fig. 2C). Using the consensus heat shock element (HSE) binding site sequence for HSF-1 (*7*, *8*) we examined the upstream promoter region of *pat-10*. We identified a strong putative HSE binding site less than 500bp from the transcription start site of *pat-10* (fig. S4). Additionally, RNAi of *hsf-1* blocked the upregulation of *pat-10* upon heat shock (Fig. 2D). Therefore, *pat-10* appears to be a direct target of HSF-1 transcriptional regulation.

Loss of *pat-10* expression resulted in reduced thermotolerance of all strains tested; we therefore tested whether ectopic overexpression of *pat-10* could be sufficient to render otherwise wild-type animals thermotolerant. Indeed, two-fold, overexpression of *pat-10* (Fig. 2E) was sufficient to significantly increase thermotolerance (Fig. 2F) and extend lifespan (Fig. 2G). Furthermore, RNAi of *pat-10* eliminated the increased thermotolerance (Fig. 2F) and lifespan (Fig. 2G) of the *pat-10* overexpression strain. Taken together, *pat-10* is necessary and sufficient for increased thermotolerance and longevity. Additionally, the beneficial effects of *pat-10* overexpression were not due to an increase in basal HSP transcription (Fig. 2H) as assayed by a GFP reporter driven by the *hsp-16.2* promoter (*9*).

One characterized function of *pat-10* is its role in the troponin complex (*10–12*), which is necessary for body wall muscle contraction. However, RNAi towards the worm homologue of tropomyosin, *lev-11*, a partner component with *pat-10* in the troponin complex, did not affect thermotolerance (Fig. 2B), while RNAi to *pat-10* or *lev-11* each result in adult onset paralysis (*11*, *13*). This suggests that the role of *pat-10* in muscle contraction is not responsible for thermotolerance.

In addition to playing a critical role in muscle contraction, loss of *pat-10* has been shown to disrupt actin cytoskeleton dynamics and endocytosis (*11*, *12*, *14*, *15*). To address these potential mechanisms of protection we first used GFP labeled muscle filaments to asses

actin organization (*16*). Upon heat shock, muscle filaments became unorganized and damaged, leading to impaired motility (Fig. 3, A and B). However, overexpression of *pat-10* was sufficient to prevent muscle and motility deterioration upon thermal stress (Fig. 3, A and B). Furthermore, we found that heat shock dramatically decreased the ratio of the filamentous (F) actin to globular (G) actin in wild-type worms, whereas the protected *pat-10* overexpression animals maintained F actin when heat stressed (Fig. 3C, fig. S5). With regard to aging, we found that the ratio of F to G actin also decreases with age and that *pat-10* overexpression lessened this decline (Fig. 3D, fig. S5). Collectively, these results suggest that under conditions of acute cellular stress or gradual age-related deterioration, the integrity of the actin cytoskeleton is fundamental to the survival of the organism and that overexpression of *pat-10* can abrogate the collapse of actin filaments.

Loss of *pat-10* via RNAi has been found in several screens to disrupt endocytosis in multiple tissues, a process dependent upon a functional actin cytoskeleton (*14*, *15*, *17*). To visualize apical endocytosis within the intestine, we soaked animals in a solution containing the fluorescent FM 4-64FX dye (*18*), which acts as a passive, late endocytic pathway marker. As expected, *pat-10* RNAi disrupted endocytosis in *C. elegans* (fig. S6). Next, to assay basolateral endocytosis, as well as quantitatively analyze the role of *pat-10*, we used a secretion and endocytosis reporter designed to actively secrete GFP (ssGFP) from muscle cells into the pseudocoelomic fluid, where it is then endocytosed by the coelomocyte cells and degraded (*19*) (fig. S7A). Therefore, the ssGFP reports upon effective secretion from the muscle cells and endocytosis by the coelomocyte; so that low GFP levels would correspond to effective secretion and uptake. Fitting with the hypothesis that overexpression of *pat-10* improves transport and cellular processing via improved subcellular scaffolding, the *pat-10 OE* strain had a decrease in overall ssGFP fluorescence (Fig. 3, E and F). The decrease in ssGFP was due to improved secretion and uptake, as seen 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. S7). RNAi of *pat-10* increased overall ssGFP fluorescence and specifically decreased muscle secretion and coelomocytic endocytosis (Fig. 3, E and G). As a further control, we found that *cup-4* RNAi, which blocks coelomocytic endocytosis (*20*), showed an even higher increase of fluorescence by blocking the uptake and degradation of ssGFP by the coelomocytes (Fig. 3G). Collectively, these data suggest that PAT-10 plays an active role in the maintenance of the cytoskeleton, which is in turn critical to cellular transport, specifically active secretion and endocytosis.

To further validate the importance of cytoskeletal integrity and endocytosis in thermotolerance, we tested whether key components in these pathways were required for survival at non-permissive temperatures. RNAi targeting a *C. elegans* homologue of cofilin, *unc-60B*, or a key regulator of coelomocytic endocytosis, *cup-4*, both reduced thermotolerance (Fig. 3H). Hence, it is clear that actin cytoskeletal maintenance and endocytosis are crucial to thermotolerance in *C. elegans*, however, we wanted to test if this mechanism is conserved.

To disrupt the actin cytoskeleton in human HEK293T cells, we used Cytochalasin D, which blocks the addition of actin monomers to filaments (*21*), or Latrunculin A, which binds monomers of actin, preventing polymerization of filaments (*22*) (Fig. 4A). Inhibiting

filamentous actin formation by either Cytochalasin D or Latrunculin A significantly reduced thermotolerance in human cell culture (Fig. 4B). Importantly, these drugs did not cause cell death when cells were unstressed (fig. S8). 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 (*23*, *24*). Because the inducible chaperone network promotes survival and proliferation of metastasizing cells (*25*), the ability to harness protective, non-chaperone components within the HSF-1 signal transduction cascade appears essential for future drug development. Our identification of *pat-10* as a modifier of thermotolerance and longevity may apply to mammalian systems without the typical oncogenic dangers associated with *hsf-1* overexpression that results in chaperone induction. An unbiased screen has already identified *pat-10* as a positive modifier of toxicity for the neurodegenerative disease protein α– synuclein (*15*). This study further demonstrated that endocytosis played a major role in neuronal dysfunction caused by α–synuclein. We suggest a similar model by which HSF-1 activity maintains cellular scaffolding and transport in times of heat stress or aging through increased *pat-10*.

The *hsf-1(CT)* strain can mount a transcriptional response to heat shock, albeit reduced in complexity to that of *hsf-1(FL)*. The molecular mechanism by which *hsf-1(CT)* regulates transcription without the C-terminal activation domain remains unclear, but possible explanations include HSF-1 containing additional activation domains. Alternatively, the *hsf-1(CT)* modification may cause affinity changes to HSE binding sites or different cofactors and coregulators, which could alter the transcriptional profile.

There is extensive evidence that molecular chaperones represent a useful defense against proteotoxic stress. Despite observations that we can enhance thermotolerance without further increasing the chaperone network, HSPs still play a pivotal role in stress resistance. In fact, small HSPs are known to interact with actin, promoting filament stability (*26–28*). Furthermore, our findings underscore the importance of maintaining filamentous actin, as opposed to the overall levels of total or globular actin. We propose a model in which HSF-1 regulates chaperones and actin cytoskeletal genes to act synergistically in a shared mechanism 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. Furthermore, a possible role for the massive induction of chaperones during heat stress is to manage the increasing pool of destabilized actin filaments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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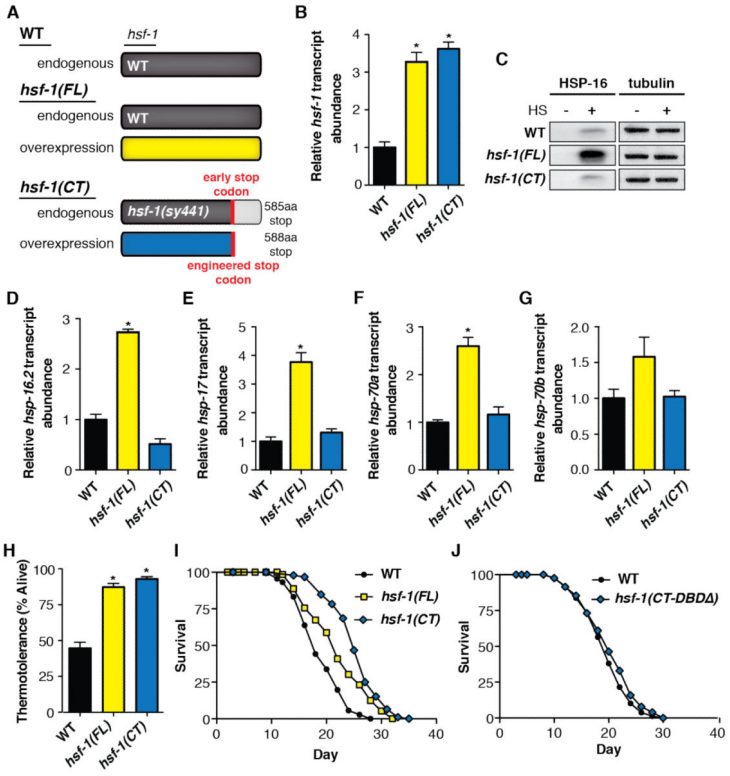

Fig. 1.

hsf-1(CT) increases lifespan and thermotolerance without enhancing the inducible chaperone network. (**A**) Diagram of *hsf-1* genotypes in wild-type (WT), full-length *(hsf-1(FL))*, and truncated (*hsf-1(CT)*) overexpression strains. (**B**) *hsf-1(FL)* and *hsf-1(CT)* equally overexpress *hsf-1*, as determined by quantitative PCR (qPCR). (**C**) Chaperone induction in *hsf-1(FL)* is enhanced, as determined by western blot of HSP-16 before and after heat shock. (**D** to **G**) qPCR of *hsp-16.2* (**D**), *hsp-17* (**E**), *hsp-70a* (C12C8.1) (**F**), and *hsp-70b* (F44E5.4) (**G**) show enhanced chaperone induction in *hsf-1(FL)*. (**H**) Thermotolerance assay of worms shifted from 20° to 34° for 13 hours. *hsf-1(FL)* and *hsf-1(CT)* survival is significantly increased. (**I**) Lifespan analysis of *hsf-1(FL)* and *hsf-1(CT)* strains show increased longevity. (**J**) Lifespan extension of the *hsf-1(CT)* strain is lost when the DNA binding domain is removed from the overexpression plasmid. *$P < 0.005$; error bars indicate SEM.

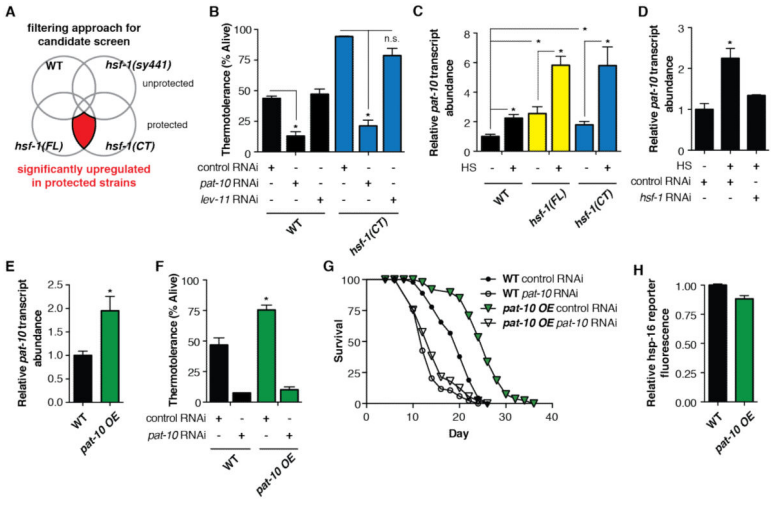

Fig. 2.

pat-10 is necessary and sufficient for thermotolerance and longevity. (**A**) Filtering selection method for RNAi based thermotolerance screen selected proteins or transcripts that were upregulated in our protected strains (*hsf-1(FL)* and *hsf-1(CT))* but not in unprotected strains (WT and *hsf-1(sy441)*), before or after heat shock. (**B**) RNAi of *pat-10* significantly reduces thermotolerance in WT and *hsf-1(CT)* strains, whereas control *lev-11* RNAi has no effect. (**C**) qPCR shows *pat-10* is upregulated by heat shock in all strains. Transcript abundance is further increased in *hsf-1* overexpression strains *hsf-1(FL)* and *hsf-1(CT)*. (**D**) Heat shock induced *pat-10* upregulation is reduced by *hsf-1* RNAi, as determined by qPCR. (**E**) qPCR of *pat-10* overexpression in the *pat-10 OE* strain. This level of overexpression is similar to the increase in *pat-10* expression after heat shock in WT worms. (**F**) *pat-10* overexpression significantly increases thermotolerance, whereas *pat-10* RNAi significantly impairs thermotolerance in WT and *pat-10 OE* strains. (**G**) *pat-10* overexpression significantly increases lifespan. *pat-10* RNAi significantly reduces longevity in WT and *pat-10 OE* strains. (**H**) qPCR shows *pat-10 OE* does not induce the *hsp-16.2*p::GFP reporter strain. **P* < 0.05; error bars indicate SEM.

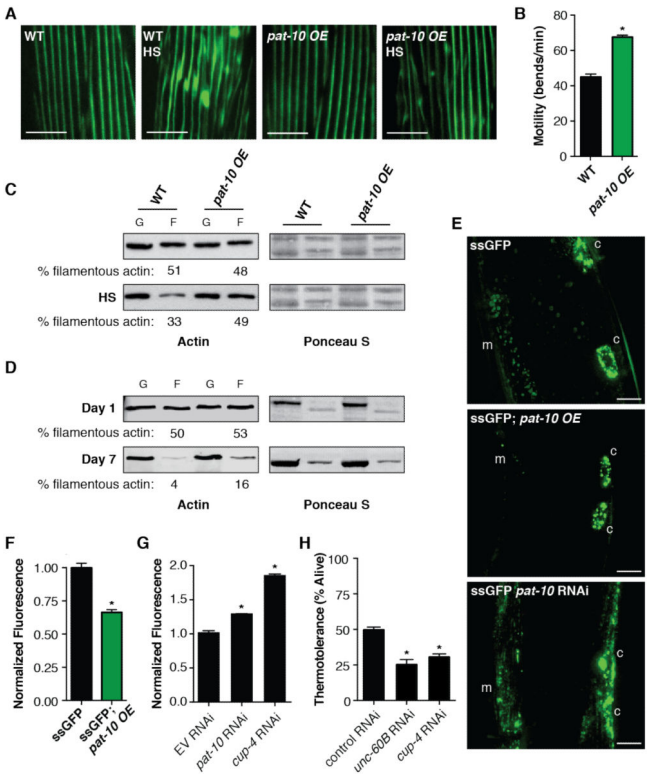

Fig. 3.

pat-10 overexpression increases actin cytoskeletal integrity and improves cellular trafficking. (**A**) GFP tagged to myosin heavy chain in muscle shows a breakdown of actin organization after heat shock in WT worms, whereas overexpressing *pat-10* maintains actin organization after heat shock. (**B**) Worm thrashes per minute in liquid were used to monitor motility. Loss of motility after heat shock is lessened in the *pat-10 OE* strain. (**C**) Heat stress causes a breakdown of filamentous (F) actin into globular (G) actin. This breakdown is prevented by overexpression of *pat-10*. Ponceau S staining shown as a loading control. (**D**) Aging causes a breakdown of F actin that is lessened by overexpression of *pat-10*. Ponceau S staining shown as a loading control. (**E**) Reporter strain that secretes GFP (ssGFP) from muscle cells (m), which is then endocytosed by coelomocytes (c) to be degraded. *pat-10 OE* strain shows increased efficiency of secretion and endocytosis, whereas RNAi of *pat-10* impairs secretion and uptake. (**F**) Normalized GFP fluorescence is significantly lower in ssGFP reporter strain overexpressing *pat-10*. (**G**) Blocking coelomocytic endocytosis increases GFP fluorescence in the ssGFP reporter strain. (**H**) RNAi of genes that block coelomocytic endocytosis decrease thermotolerance. **P* < 0.05; error bars indicate SEM; scale bars indicate 10 μm.

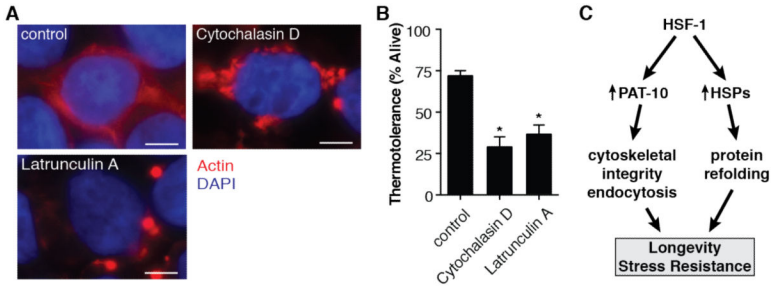

Fig. 4.

Impairing actin dynamics decreases thermotolerance in mammalian cell culture. (**A**) HEK293T cells treated with Cytochalasin D or Latrunculin A show disrupted filamentous actin formation in mammalian cell culture. (**B**) Both Cytochalasin D and Latrunculin A treatment reduce the thermotolerance of HEK293T cells after a heat shock of 45°C for 2 hours. (**C**) Proposed model of the duel pathways of HSF-1 mediated health assurance. **P* < 0.05; error bars indicate SEM; scale bars indicate 5 μm.