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REPORT

Costs, benefits and redundant mechanisms of adaption to chronic low-dose stress in yeast

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ABSTRACT

All organisms live in changeable, stressful environments. It has been reported that exposure to low-dose stresses or poisons can improve fitness. However, examining the effects of chronic low-dose chemical exposure is challenging. To address this issue we used temperature sensitive mutations affecting the yeast cell division cycle to induce low-dose stress for 40 generation times, or more. We examined *cdc13-1* mutants, defective in telomere function, and *cdc15-2* mutants, defective in mitotic kinase activity. We found that each stress induced similar adaptive responses. Stress-exposed cells became resistant to higher levels of stress but less fit, in comparison with unstressed cells, in conditions of low stress. The costs and benefits of adaptation to chronic stress were reversible. In the *cdc13-1* context we tested the effects of Rad9, a central player in the response to telomere defects, Exo1, a nuclease that degrades defective telomeres, and Msn2 and Msn4, 2 transcription factors that contribute to the environmental stress response. We also observed, as expected, that Rad9 and Exo1 modulated the response of cells to stress. In addition we observed that adaptation to stress could still occur in these contexts, with associated costs and benefits. We conclude that functionally redundant cellular networks control the adaptive responses to low dose chronic stress. Our data suggests that if organisms adapt to low dose stress it is helpful if stress continues or increases but harmful should stress levels reduce.

Introduction

All organisms, from single celled bacteria and yeasts to complex mammals, experience a variety of environmental conditions, many of which are stressful. Organisms have evolved to adapt to different stresses and to thrive in varying conditions.[1] Experiments in bacteria, yeast, worms, fish and mammals have revealed that different types of stress each induce common responses, termed the environmental stress response (ESR) or the cellular stress response (CSR).[2-8] Responses induced by the ESR/CSR affect oxidative stress responses, protein folding, protein degradation, carbohydrate and fatty acid metabolism, DNA damage responses and intracellular signaling.[3,9-11] Organisms exposed to stress often become resistant to higher levels of the same stress and cross-resistant to other stresses.[8,12-15] The evidence is that a complex network of interactions regulates general as well as more specific responses to stress.[16-18]

There is evidence that low-level stress can improve aspects of organismal fitness. In 1888 it was reported that yeast cells grew better when exposed to low-doses of poisons, such as arsenic trioxide, than in the absence of poison.[19] This type of dose-response relationship, in which exposure to low-doses of a "poison" (e.g. environmental factor or chemical agent) improves some aspect of the fitness of an organism, is known today as hormesis. Hormesis has been reported in a variety of
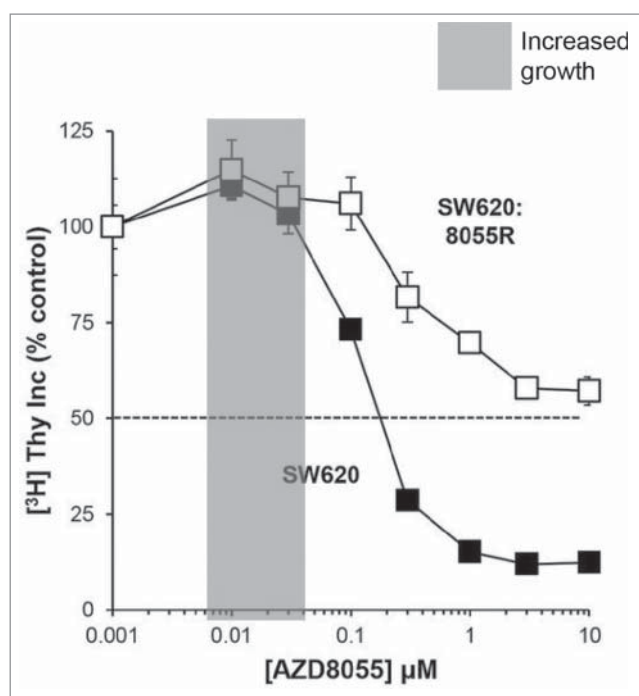
different systems from bacteria to yeast to vertebrates.[20-27] A hormesis type response can often be observed in human cells growing in culture. Figure 1, adapted from Cope *et al.*,[26] shows an example where growth of 2 human cancer cell lines, as measured by tritiated thymidine incorporation, is increased by low-doses of a Tor kinase inhibitor (Fig. 1).[26] Numerous other studies with different biological end points, such as the effects of gamma radiation on mouse tumor formation or of DNA damage on worm longevity, support the idea that low-dose stresses can have a positive effect on organism fitness.[20,21, 25,27]

It is suggested that the biochemical mechanisms that explain hormesis are adaptive stress responses.[28-30] Any amount of extrinsic stress will cause harm, for example by damaging DNA or other cellular components. However, it is thought up-regulation of maintenance and repair systems, in response to low-dose stress, protects not only from the extrinsic stress but also from intrinsic stresses that affect carcinogenesis, aging or other aspects of organismal fitness.[31-33] If so, then perhaps it is mild induction of the ESR/CSR that explains the hormesis response.[14,34] However, upregulation of maintenance and repair systems must come at some type of metabolic cost, otherwise organisms would never reduce maintenance and repair levels.[1] These arguments suggest that hormesis is unlikely to occur very frequently.

Figure 1. Hormetic response to mild stress in human cell lines. Human SW620 and SW620:8055R cell lines, the parental sensitive and a derived resistance line, were exposed to increasing concentrations of TOR kinase inhibitor, AZD8055, for 24 hours. Proliferation was assayed by [³H]thymidine incorporation. This figure is based on Figure 2 A of Cope *et al.*[26]. We added a gray box to highlight the area of increased fitness.

When measuring the positive or negative effects of low-dose stress it is important to carefully consider what end point is measured. For example, mutations that extend lifespan in worms, and are considered positive by this criterion, also dramatically reduce the ability of worms to produce offspring in the laboratory environment and therefore have clear negative effects too.[35,36] Moreover, the biochemical mechanisms underlying any positive responses to low dose stresses are poorly understood.[37-39] The lack of good biochemical understanding of the response to chronic low-dose stresses or poisons stems, in part, because the effects induced are small and difficult to measure.[21,40] Another difficulty, particularly for studies on the effects of chemicals, is that low-dose impurities, or chemical break down products, might confound interpretation. For example, at low concentrations the poison methotrexate improves viability of breast cancer cell lines.[41] If methotrexate were contaminated with the chemically similar growth promoter, folate, then this might make interpretation of any positive effects of methotrexate difficult to distinguish from the effects of lower concentrations of the contaminant folate.

In this paper we carefully examined the responses of budding yeast, *Saccharomyces cerevisiae*, to chronic low-dose stress. Budding yeast is perhaps the best-studied model organism and much is known about its responses to stress.[42] Although growth of yeast in the laboratory is artificial, for example with unlimited food supply or constant temperature, laboratory growth conditions mimic part of the natural yeast life cycle. It is thought that when yeast cells arrive on fruit, after being carried there by wasps, they divide rapidly from single cells.[43] For this reason the rapid growth of yeast cultures in the laboratory is a biologically relevant measure of fitness.

To study the effects of chronic stress in yeast we used genetic mutations, rather than chemicals, to poison different aspects of the cell cycle. Temperature sensitive *cdc* (**c**ell **d**ivision **c**ycle) mutations affecting different aspects of cell cycle progression were used.[44,45] We considered *cdc* mutations particularly attractive tools for this purpose because the dose of stress can be simply adjusted by controlling the culture temperature. The higher the temperature the more "poisoned" cell cycle events become. Furthermore, since each cell in the population carries the same mutation we can be sure that each cell in the environment is stressed to a similar extent. *cdc13-1* mutants are defective in telomere related functions. At high temperatures telomeres of *cdc13-1* cells induce a DNA damage response, akin to the response to DNA double strand breaks elsewhere in the genome.[46,47] In this sense, the effect of *cdc13-1* mutation mimics that of genotoxic agents. Temperature sensitive *cdc15-2* mutants are defective in a kinase required for exit from mitosis and arrest cell division in late anaphase at high temperatures.[48] Using these yeast genetic tools we asked: Does adaptation to chronic low-dose stress have a positive effect, a negative effect, neither, or both? We also addressed whether adaptation to stress is reversible and dependent on specific pathways.

Results

cdc13-1 *mutants adapt to chronic telomeric stress*

To examine the response of yeast cells to chronic low-dose of telomere stress we used strains containing the *cdc13-1* allele, affecting the essential telomere capping protein Cdc13. *cdc13-1*, a point mutation, causes a P371S amino acid change and this induces temperature dependent Cdc13-1 protein degradation.[49,50] In our lab *cdc13-1* strains are routinely cultured at 23°C (a permissive temperature). However, *cdc13-1* shows synthetic genetic interactions with mutations affecting the KU or MRX complexes at this temperature and thus we know that Cdc13-1 is not fully functional at 23°C.[51,52] Therefore we also sometimes culture *cdc13-1* strains at 20°C, where Cdc13-1 is more functional. At higher temperatures *cdc13-1* mutants have dysfunctional telomeres, generate long telomeric 3′ ssDNA G-tails and activate Rad9-dependent cell cycle arrest.[46,53] We have previously reported that *cdc13-1* mutants cultured at 30°C, a normal temperature for growing yeast, induce the ESR.[54]

It is known that many recessive, loss of function mutations, for example affecting DNA damage response (DDR) or nonsense mediated RNA decay (NMD) pathways, improve the fitness of *cdc13-1* strains grown at >26°C.[55,56] Therefore we performed experiments in diploid cells to reduce the chance that recessive loss of function mutations affecting DDR, NMD or other genes were selected during our experiments. We passaged *cdc13-1* cells at 23°C, as usual, or at 25°C, a slightly higher temperature, to induce chronic low-dose telomere stress. 25°C slightly reduced the fitness (colony size) of *cdc13-1* mutants grown on agar plates, and therefore 25°C was considered the maximum permissive temperature[46] (Supplementary Figure 1A). Interestingly, there was heterogeneity in colony size after the first passage at 25°C, but this disappeared by the second passage (Fig. 2B). This suggests that all cells in the population have adapted to the increased stress by the second passage.
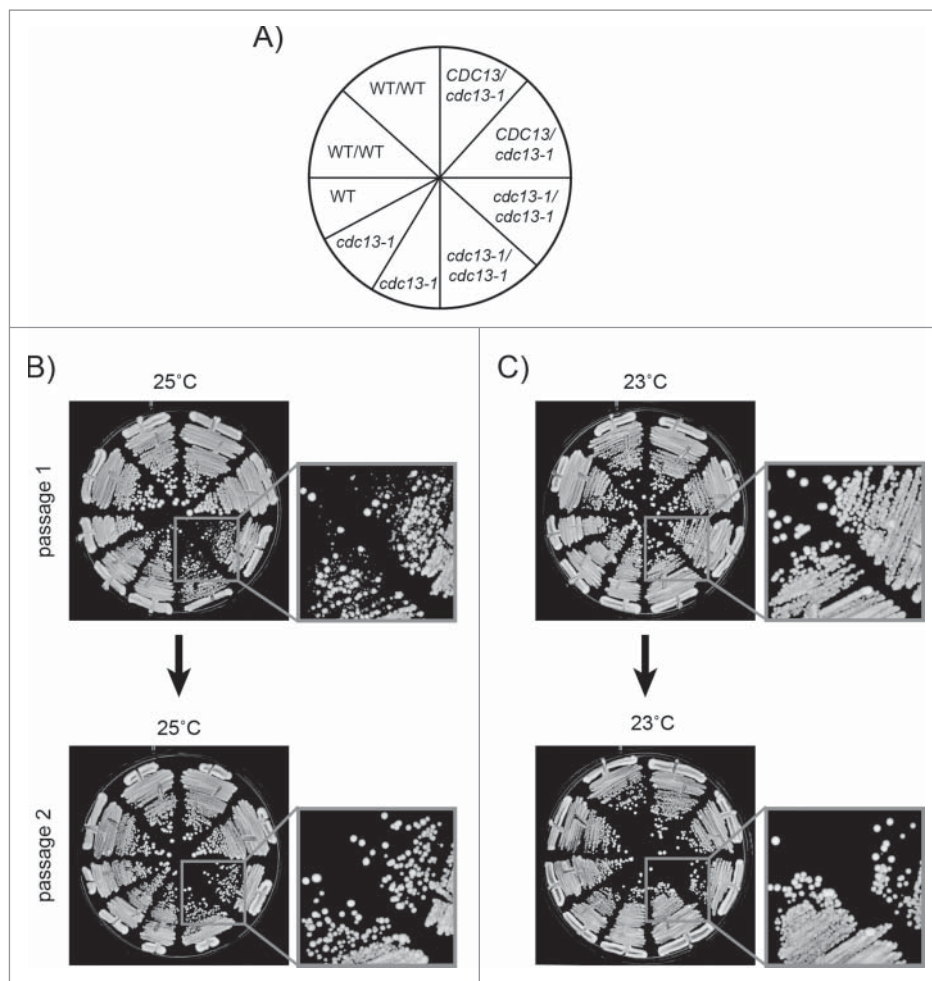
Figure 2. Passaging procedure. Freshly unfrozen strains were patched on YEPD agar plates and incubated at 23°C for 3 days, then passaged at 23°C or 25°C on YEPD agar plates as shown. 5–10 colonies from each genotype were pooled at each passage. Strains were: DDY81, 739, 738, 737, 736, 735, DLY1108, 1195, 3001. A) Genotypes of strains streaked on agar plates. B) Cells passaged for 2 passages at 25°C. C) Cells passaged for 2 passages at 23°C. DDY strains are diploids. DLY strains are haploids.

The heterogeneity in colony size was not observed when cells were passaged at 23°C (Fig. 2C).

Importantly, we never observed, at any temperature or time, that *cdc13-1* cells grew better than *CDC13* cells grown on the same plate (Fig. 3). By this criterion we see no evidence for a hormetic effect in this experimental system. In other words we see no evidence that exposure to chronic low-dose telomere stress improved fitness. However, we did observe that *cdc13-1* strains previously passaged at 25°C grew better at 26°C or 27°C than genetically identical strains passaged at 23°C (Fig. 3). We conclude that, as expected, adaptation to chronic mild telomere stress improves the fitness of strains exposed to even higher levels of telomere stress.

Interestingly, we observed that *cdc13-1* strains, previously passaged at 25°C, grew less well at 20°C than genetically identical strains previously passaged at 23°C. This phenomenon is telomere stress related, rather than temperature stress related, since equivalent *CDC13* cells, previously passaged at 23°C, were fully fit at 20°C. The simplest interpretation of these data is that the adaptation to telomere stress caused by growth at 25°C comes at a cost (trade off) affecting growth rate when telomere stress is reduced. Thus, in this context, adaptation to chronic telomere stress reduces fitness and is, in a sense, the opposite of hormetic.

Finally we tested whether the positive and negative effects of passaging *cdc13-1* cells at 25°C were reversible. Cells grown for 7 passages at 25°C were grown for a further 3 passages at 23°C before fitness was assessed. At 26°C such cells exhibited an intermediate growth phenotype, somewhere between the fitness of strains previously grown at 23°C or 25°C. Thus adaptation to telomere stress seems to be at least partially reversible. As predicted the haploid *cdc13-1* strain cultured at 25°C and then at 23°C did not reverse its ability to grow well at 26°C and above. Presumably this strain accumulated genetic mutations during growth at 25°C that suppressed *cdc13-1* defects. Interestingly, the cost of adaptation to growth at 25°C, seen at 20°C, seems to be fully reversed by growth for 3 passages at 23°C. Again, therefore, adaptation seems to be reversible and is, by this criterion, epigenetic rather than genetic.

Adaptation to low-level of chronic telomeric stress is a robust response

Budding yeast responses to *cdc13-1* induced telomere defects are complex and have been well studied.[45,46, 54,56, 57] We tested whether removal of Rad9, a central player in the response to *cdc13-1* induced telomere defects, affected adaptation. Rad9 was the first DNA damage checkpoint protein to be classified
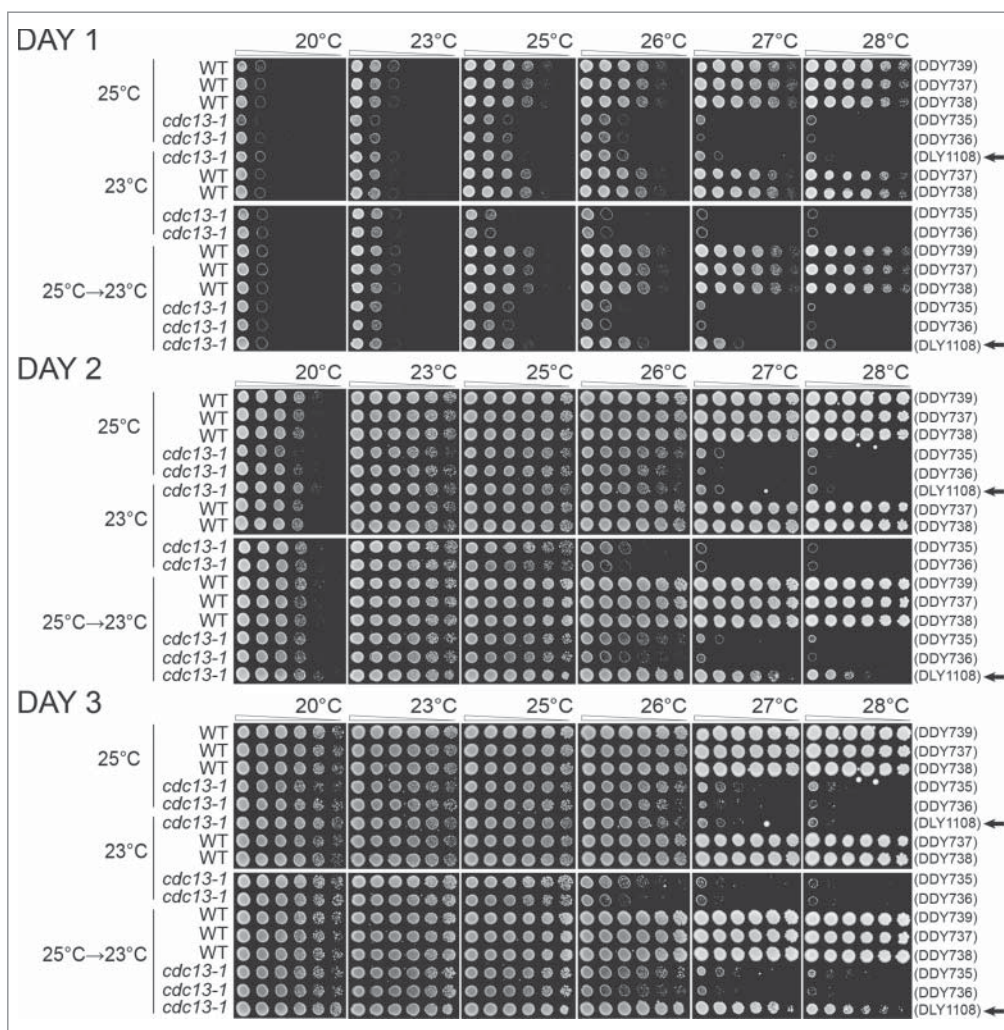
Figure 3. The effects of adaptation to low-level of telomere stress in *cdc13-1* mutants. Diploid strains with the genotypes indicated were grown on solid agar plates for 10 passages at 23°C, 10 passages at 25°C, or for 7 passages at 25°C followed by 3 passages at 23°C as indicated on the left of the Figure. Strains (DDY735, 736, 737, 738, 739 and DLY1108) were then inoculated into liquid and grown overnight at 23°C or 25°C. Fivefold dilution series of strains were set up in 96 well plates transferred to several independent solid YEPD agar plates using a pin tool. Individual plates were incubated at different temperatures, indicated across the top of the Figure, and photographs taken after 24, 48 and 72 hours of incubation. Arrows on right indicate haploid cultures.

and plays at least 3 critically important roles in *cdc13-1* mutants. Rad9 is essential for cell cycle arrest of *cdc13-1* cells in metaphase, to inhibit ssDNA production near uncapped telomeres, and to maintain cell viability.[53,58-60] Using temperature sensitive *cdc13-1 rad9Δ* mutants we performed analogous experiments to those performed on *cdc13-1* mutants. The maximum permissive temperature of *cdc13-1 rad9Δ* mutants is about 26°C, higher than *cdc13-1* mutants, presumably because low-levels of telomere damage induced at 26°C are sufficient to activate Rad9-dependent checkpoint pathways (inducing metaphase arrest), but insufficient to cause much cell death (as judged by colony size on plates) (Supplementary Figure 1A).

Interestingly, we observed that *cdc13-1 rad9Δ* strains adapted after 2 passages at 26°C, similarly to *cdc13-1* strains at 25°C (Fig. 4A-B, Supplementary Figure 2). That is, *cdc13-1 rad9Δ* strains previously grown at 26°C grew better at higher temperatures, 28°C or 29°C, than genetically identical cells grown at 23°C (Fig. 4B). However, such cells exhibited poorer growth at lower temperatures, 20°C and 23°C. If *cdc13-1 rad9Δ* mutants were moved back from 26°C to 23°C, then the effects of adaptation were partially reversed. We conclude that *cdc13-1 rad9Δ* strains,

like *cdc13-1* strains, can adapt to telomere damage and that the adaptation is reversible and comes with costs and benefits.

We also tested adaptation in the absence of Exo1, an exonuclease that degrades defective telomeres in *cdc13-1* strains.[61] The maximum permissive temperature of *cdc13-1 exo1Δ* mutants is also about 26°C (Supplementary Figure 1B). Similarly to what was observed in *cdc13-1 rad9Δ* strains, we observed that *cdc13-1 exo1Δ* strains adapted after 2 passages at 26°C and grew better at 27°C and 28°C but exhibited poorer growth at lower temperatures, 20°C and 23°C (Fig. 4C, Supplementary Figure 3). When moved back from 26°C to 23°C, the effects of cells' adaptation were partially reversed. We conclude that *cdc13-1 exo1Δ* strains, like *cdc13-1* or *cdc13-1 rad9Δ* strains, can adapt to telomere damage and that the adaptation is reversible and comes with costs and benefits.

Finally we tested adaptation in the absence of Msn2/Msn4, 2 transcriptional activators that underlie the transcriptional response to numerous stresses.[2,62-65] The maximum permissive temperature of *cdc13-1 msn2Δ msn4Δ* mutants is about 25°C (Supplementary Figure 1C). *cdc13-1 msn2Δ msn4Δ* strains adapted to 25°C after 2 passages, and grew better at 26°C, but
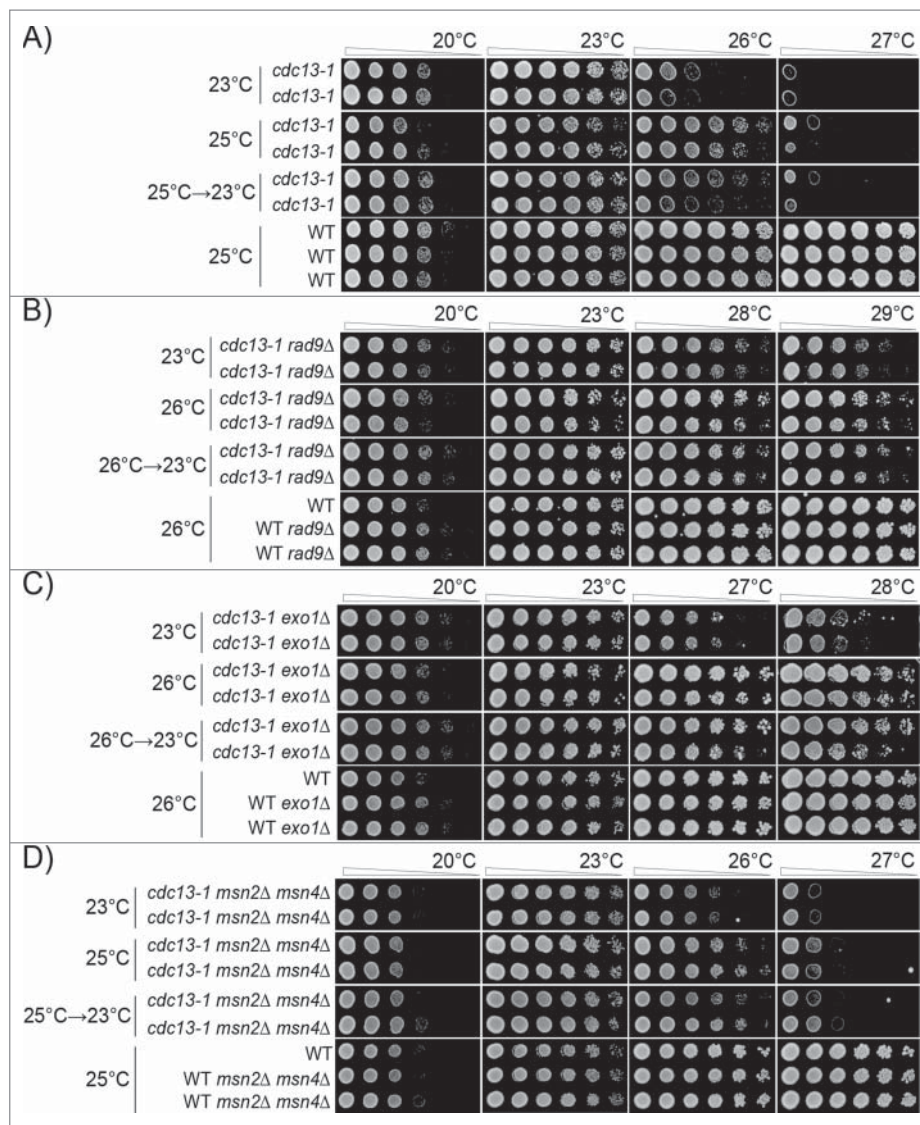
Figure 4. Adaptation to telomere stress in the absence of key stress response proteins. Diploid strains of different genotypes were analyzed as in Figure 3 except that strains were passaged twice at high temperature (25°C or 26°C), or once at high temperature followed by a single passage at low temperature, before being spotted onto agar plates. A) Strains as in Figure 3. Strains were DDY735, 736, 737, 738 and 739. B) Strains were DDY739; 868, 869, 860 and 861. C) Strains were DDY739, 992, 993, 994 and 995. D) Strains were DDY739, 974, 975, 972, 973. All strains shown in each subsection, at each temperature, were grown on single agar plates but images have been cut and pasted to make comparisons easier. Images were taken at 48 hours of incubation. Images taken after 24, 48 and 72 hours incubation are shown in Supplementary Figures: 2, 3 and 4, respectively.

exhibited poorer growth at lower temperatures, 20°C and 23°C (Fig. 4D, Supplementary Figure 4). As before, the effects of adaptation were partially reversed when cells were returned from 25°C to 23°C (Fig. 4D).

In summary Figure 4 clearly shows that loss of critically important components of the DNA damage or stress induction networks, active in *cdc13-1* strains, still permits *cdc13-1* strains to adapt to stress. Thus we conclude that adaptation to chronic telomere-induced stress involves a robust network of responses.
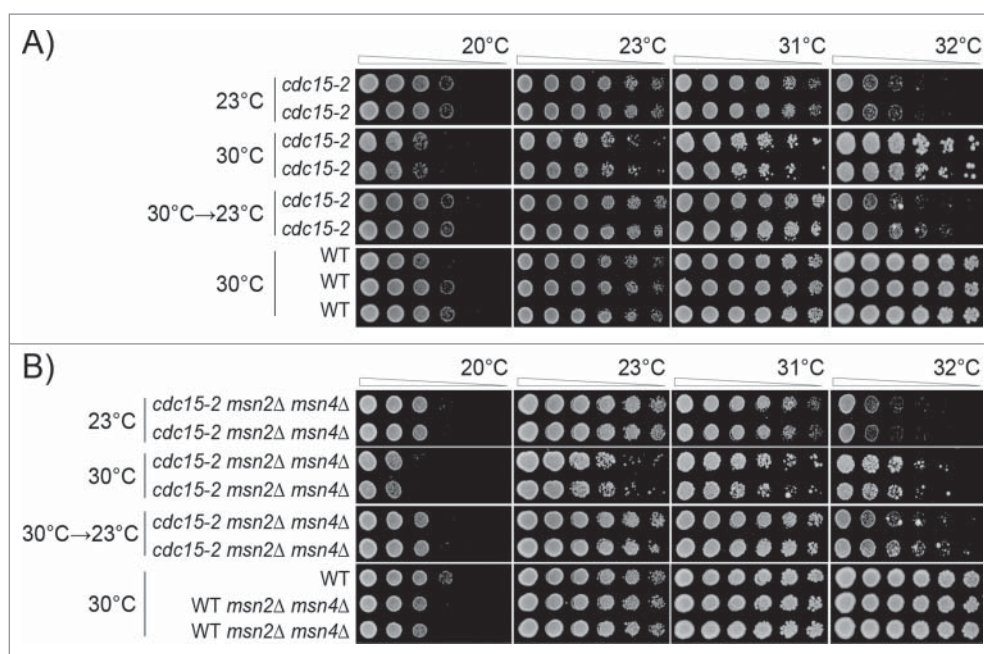
Adaptation to mitotic kinase inhibition

Mammalian cells exposed to low-doses of Tor kinase inhibitor grow better than cells not exposed to the inhibitor (Fig. 1).[26] We therefore extended our genetic studies to examine responses to low-level inhibition of kinase activity. We chose to examine mutations affecting the Cdc15 kinase, not a member

of the Tor kinase family, but which is an essential component of the Mitotic Exit Network and is critical for the completion of mitosis.[66,67] Using temperature sensitive *cdc15-2* mutants we performed analogous experiments to those performed in telomere defective *cdc13-1* mutants. Since the maximum permissive temperature of *cdc15-2* mutants is about 31°C, the temperature used to cause mild stress was 30°C (Supplementary Figure 1A, D). We determined that we could observe adaptation to growth at 30°C after as little as 3 passages (Fig. 5A, Supplementary Figure 5).

We could draw very similar conclusions about how cells adapt to *cdc15-2* induced kinase defects as we had drawn earlier for *cdc13-1* mutants. We never observed, at any temperature or time, that *cdc15-2* cells grew better than *CDC15* cells grown on the same plate (Fig. 5A, Supplementary Figure 5). By this criterion we see no evidence for a hormetic effect in *cdc15-2* strains. Analysis of *cdc15-2* mutants showed clear evidence for

Figure 5. Adaptation to low-level kinase inhibition in *cdc15-2* mutants. Diploid strains of different genotypes were analyzed as in Figure 3. Strains were grown for 3 passages at 23°C or 30°C on agar plates, or for 1 passage at 30°C and 2 at 23°C. A) Strains were DDY739; 839, 840, 841, 842. B) Strains were DDY739; 1036, 1037 1038, 1039. All strains shown in each subsection, at each temperature, were grown on single agar plates. Images taken after 24, 48 and 72 hours incubation are shown in Supplementary Figures: 5 and 6, respectively.

adaptation to kinase inhibition, a clear cost of adaption to kinase inhibition and strong evidence that adaptation was reversible (Fig. 5A). In fact, adaptation to *cdc15-2* stress seemed nearly fully reversible after 2 passages at 23°C (Fig. 5A).

We also examined the effects of Msn2/Msn4 in the context of Cdc15 defects. *cdc15-2 msn2Δ msn4Δ* mutants adapted after 2 passages to 30°C, to exhibit better growth at 31°C and 32°C but poorer growth at lower temperatures, 20°C and 23°C (Fig. 5B, Supplementary Figure 6). The effects of adaptation were partially reversed when moved from 30°C to 23°C (Fig. 5B).

Discussion

We analyzed yeast mutants defective in the cell division cycle to measure the costs and benefits of adapting to chronic low-level stress. We were particularly interested to determine if we could find evidence for hormesis, which has been defined as "a low-dose stimulation or beneficial effect and a high dose inhibitory or toxic effect,"[23] in this simple model system. Figure 6 summarizes our observations and shows the positive and negative effects of adapting to different types of low-dose stress. We observe that previous exposure to low-dose stress improves the ability of yeast to survive higher doses of the same stress (Fig. 6, position B). However, the trade-off for adapting to low-dose stress is that cells are less fit, in comparison with unstressed cells, when grown in low stress conditions (Fig. 6, position A). We see no evidence for hormesis in this experimental system, that is that yeast cells exposed to low-dose stresses are fitter than cells not exposed to stress (Fig. 6, the predicted hormesis response curve, above the black dashed line). We cannot, of course, exclude that hormesis occurs in response to other types of stress, or could be observed if other biological end points were measured. For example, if we measured genetic stability

rather than growth rate we might observe that cells with a slight induction of the DNA damage response were more stable than cells that had not induced the response.

What mechanisms might explain the different effects of the adaptive response to low-dose stress? *cdc13-1* and *cdc15-2* affect different aspects of cell cycle progression, but we imagine the types of adaptive response are somewhat similar. Defective telomeres in *cdc13-1* mutants induce a DNA damage checkpoint kinase dependent network, involving at least 5 kinases, Mec1,
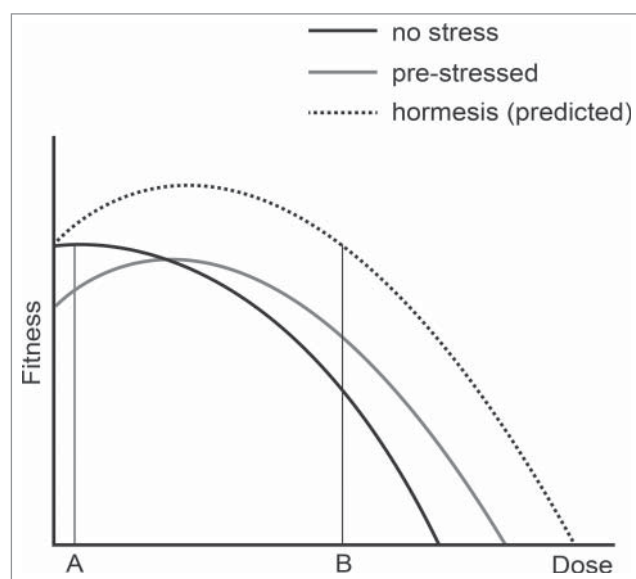
Figure 6. The effects of adaptation to mild stress. Three curves are used to represent the fitness of yeast cells that were previously not stressed (black line), pre-stressed (gray line) or show a hormetic response (dashed line). A and B indicate low and high doses of stress.

Tel1, Chk1, Rad53 and Dun1 to affect cell cycle arrest and DNA repair. Therefore effects on phosphorylation-dependent biochemical processes such as signaling, transcription and proteolysis must underlie the adaptive responses. Rad9, for example, is essential for cell cycle arrest of *cdc13-1* strains, for activating downstream kinase activities and for inhibiting the accumulation of single stranded DNA near telomeres.[47,53, 58,68-73] Furthermore rearrangements in telomere DNA structure can also reduce cellular dependence on Cdc13.[74]

Since adaptation can occur in *cdc13-1 rad9Δ, cdc13-1 exo1Δ* and *cdc13-1 msn2Δ msn4Δ* strains, we conclude that much redundancy exists in the responses to telomere defects and that a strong network structure must underlie the adaptive responses. Consistent with this Berry et al.[16] have shown acquired stress resistance is complex and context dependent. The fact that adaptation can occur in the absence of Rad9, which plays so many different and important roles in telomere defective strains, shows how robust (and presumably important) this adaptive response is. The complex network structure that underlies adaption may explain that numerous mechanisms have been proposed to account for hormetic mechanisms.[28-30, 37-39] We have not examined the mechanisms that control adaptation to *cdc15-2* defects but we imagine that these mechanisms are equally as robust since Cdc15 is part of the mitotic exit network. We found that cells adapting to *cdc13-1* or *cdc15-2* induced stress incurred a detectable cost to fitness when the stress was reduced. Furthermore, cells seemed to reverse adaptation to *cdc15-2* stress more rapidly that they reversed adaptation to *cdc13-1* stress. This comparison suggests that the each type of stress can induce quite different adaptive mechanisms.

All organisms live in stressful, variable environments, encountering harmful physical and chemical stressors such as sunlight, radiation and oxygen. All of these poisons are unavoidably dangerous and cells have evolved powerful mechanisms to overcome most, but not all, of their harmful effects. It is likely that complex networks of biochemical interactions help cells tolerate and thrive in the context of these different stresses. Presumably, therefore, *cdc* mutants growing at slightly elevated temperatures up-regulated responses that counteract the negative cues caused by *cdc* defects. This up regulation, or adaptation, is positive because it prepares cells for exposure to even more stress. On the other hand adaptation is harmful when the stress is removed. In a sense *cdc* adapted cells have become "addicted" to stress, presumably because the energetic cost of maintaining a stress response when stress is no longer present reduces fitness.

In animals too there seems to be both a benefit and a cost of adapting to stress. Zebra finches exposed to heat stress in early life are more tolerant of heat later in life, but have shorter life-spans than unstressed birds if no further stress is received.[75]

cdc13-1, which essentially damages chromosomes, can be considered as a model for environmental stresses that cannot be avoided, such as radiation and oxygen exposure. We routinely grow *cdc13-1* cells at 23°C, although we know that telomeres of *cdc13-1* mutants at 23°C are imperfect, because synthetic lethal interactions are seen in combination with *yku70Δ, yku80Δ, mre11Δ, rad50Δ* and *xrs2Δ* mutations at this temperature.[51,52] Presumably, therefore, the *cdc13-1* strains growing at 23°C have adapted to growth with defective telomeres. Along similar lines, most mammalian cells are grown in the laboratory in 20%

oxygen, yet when cells have adapted to grow in lower oxygen concentrations and are switched back to 20% oxygen, then a strong stress response is observed.[76-78] Similarly, all organisms are exposed to low-levels of radiation, but exposure can be increased environmentally. Interestingly, the most recent studies show that comparatively small radiation exposure in radiation workers leads to increased risk of death from leukemia.[79,80] Our experiments measured growth rate, as a measure of fitness, but of course there are many other relevant measures of organism fitness. For example, it is likely that if we were to measure genetic stability of *cdc13-1* or *cdc15-2* cells grown at high temperature then stability would be reduced.

Experiments on mammalian cells in culture clearly show hormetic type responses to stress,[26] so why do we not see evidence for hormesis in yeast? We favor the explanation that culture of transformed cancer cells in the laboratory does not reflect the carefully regulated cell growth that occurs in an organism. In tissue culture, perhaps, slightly inhibiting growth promoting signal transduction pathways (e.g., TOR) actually improves the fidelity of cell division and increases fitness. We suggest that the yeast experimental system is a better model for real organism growth. We cannot exclude, of course, that with a different experimental set up we might see hormesis in yeast, as was apparently seen in 1888.[81]

If organisms could be truly fitter after exposure to a toxin/poison/stress, then natural selection would surely select for organisms that generated or mimicked their own toxin/poison/stress. Environments change rapidly and it is not possible to predict what stresses are in store for the future. Our data suggests the idea that responses to chronic low-dose stress are not universally positive. The good news is that organisms can, if necessary, adapt to stress and this helps them survive increased stress. The bad news is that there is a price to be paid if the stress reduces. It has been suggested that positive responses to low-doses poisons will protect humans against cancer and/or aging.[21,27] We caution against this view.

In summary our analyses of yeast cells exposed to chronic low-level telomere stress or mitotic kinase inhibition show clear evidence for adaption to stress. Adaptation improves cell fitness if cells are exposed to more of the same stress, but reduces fitness when stress levels are reduced. We found that adaptation to stress is reversible and that functionally redundant, robust networks of biochemical interactions must be responsible for the adaptive responses. Our results suggest that adaptation to low-level stress is favorable should stress remain or increase, but harmful when stress levels reduce.

Materials and methods

Yeast strains used in this study

All *S. cerevisiae* strains used in this study are in the W303 genetic background: *ade2-1 can1-100 trp1-1 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5+* and are shown in Supplementary Table 1.

Media

Strains contain an *ade2-1* mutation and therefore the liquid or solid medium was supplemented with adenine (1L YEPD: 10g

yeast extract, 20g bactopeptone, 50ml 40% dextrose, 15ml 0.5% adenine, 935ml H_2O).

Cell passage

Yeast strains were passaged on YEPD agar plates by streaking by toothpick at the indicated temperatures. Between 5 to 10 colonies from each genotype were pooled at each passage. We counted the cells in representative colonies and estimate that for each passage, for a single cell to form a colony represents approximately 21 cell generations.

Spot test assays

Pooled colonies from cell passage plates were inoculated into 2ml liquid YEPD and grown on a wheel for 12-18 hours to reach saturation. Saturated liquid cultures were 5-fold serially diluted in sterile water in 96-well plates, and small aliquots were spotted onto YEPD plates with a sterile, metal pin-tool. Plates were incubated at the temperatures indicated and photographed at 24, 48 and 72 hours.

Image processing

All agar plate images were processed using Adobe Photoshop CS6 software. In each Figure, all images were adjusted identically; there were slight differences across Figures. Images were adjusted as follows: in the Levels adjustment tool the low end of the intensity histogram was trimmed to between 100–130 and all other values left at default (1, 255). Using Photoshop's exposure tool, Exposure settings were adjusted to 0.3–0.8; Offset to −0.02−−0.05; and Gamma Correction to 1.2–1.5. Color was converted to grayscale after import into Adobe Illustrator.

Abbreviations

cdc   cell division cycle
ESR   environmental stress response
CSR   cellular stress response
DDR   DNA damage response
NMD   nonsense mediated RNA decay

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

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