RESEARCH ARTICLE

End-of-life cell cycle arrest contributes to stochasticity of yeast replicative aging

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Abstract

There is growing evidence that stochastic events play an important role in determining individual longevity. Studies in model organisms have demonstrated that genetically identical populations maintained under apparently equivalent environmental conditions display individual variation in life span that can be modeled by the Gompertz–Makeham law of mortality. Here, we report that within genetically identical haploid and diploid wild-type populations, shorter-lived cells tend to arrest in a budded state, while cells that arrest in an unbudded state are significantly longer-lived. This relationship is particularly notable in diploid BY4743 cells, where mother cells that arrest in a budded state have a shorter mean life span (25.6 vs. 35.6) and larger coefficient of variance with respect to individual life span (0.42 vs. 0.32) than cells that arrest in an unbudded state. Mutations that cause genomic instability tend to shorten life span and increase the proportion of the population that arrest in a budded state. These observations suggest that randomly occurring damage may contribute to stochasticity during replicative aging by causing a subset of the population to terminally arrest prematurely in the S or G2 phase of the cell cycle.

Introduction

Aging is an inherently stochastic process. Even among genetically homogeneous populations under nearly identical environmental conditions, there is variation in individual longevity and health span (Herndon *et al.*, 2002; Kirkwood *et al.*, 2005; Rea *et al.*, 2005; Pincus *et al.*, 2011). The budding yeast *Saccharomyces cerevisiae* provides a useful model system for studying the genetic and environmental factors that underlie stochasticity of aging.

In the yeast replicative aging paradigm, replicative life span (RLS) is defined as the number of daughter cells produced prior to irreversible cell cycle arrest under nutrient replete conditions (Mortimer & Johnston, 1959; Kaeberlein, 2010). Similar to other model organisms, genetically identical yeast within a population do not senescence at the same age (Kaeberlein *et al.*, 2001). Replicative senescence in yeast typically occurs in the G1 phase of the cell cycle, where cells arrest in the unbudded state; however, even in genetically homogenous yeast populations, a significant proportion (often 40% or higher) of yeast arrest with small or large buds, or in some cases with a cluster of interconnected cells(Pichova *et al.*, 1997; Nestelbacher *et al.*, 1999; McVey *et al.*, 2001; Merker & Klein, 2002; Falcon & Aris, 2003). In yeast, bud emergence occurs at the beginning of S phase (Hartwell *et al.*, 1974; Di Talia *et al.*, 2007), suggesting that cells that have

a budded terminal morphology have failed to exit the cell cycle in G1 and have progressed into S phase.

Determining the terminal morphology of yeast mother cells is experimentally straightforward and can be easily accomplished by microscopic visualization of cells at the end of the RLS experiment. Despite the experimental simplicity of the assay, there is currently only limited data available on this subject, comprising terminal morphology data for a total of approximately 250 wild-type mother cells across three strain backgrounds (Table 1). The relative lack of data in this area likely stems from the fact that tracking terminal morphology of individual yeast mother cells is a labor intensive process generally requiring full RLS analysis involving manual micromanipulation of daughter cells from mother cells (Steffen *et al.*, 2009). For this reason, prior studies have identified only a few specific mutations that alter the proportion of cells with unbudded or budded terminal morphologies. For example, deletion of the RecQ helicase gene, *SGS1*, increases the proportion of budded cells at senescence (Gangloff *et al.*, 2000; Johnson *et al.*, 2001). Deletion of the gene encoding the RNA polymerase II component Hpr1 also results in a similar phenotype (Merker & Klein, 2002). These two genes share a function in promoting genomic stability at the ribosomal DNA cluster and elsewhere, and both mutants also have reduced RLS (Sinclair *et al.*, 1997; Defossez *et al.*, 1999; Merker & Klein, 2002).

We have recently begun collecting terminal morphology data for individual mother cells as part of an ongoing effort to quantify replicative life span for each of the viable haploid strains in the yeast ORF deletion collection (Kaeberlein & Kennedy, 2005; Kaeberlein *et al.*, 2005a, b). In the wild-type background of the yeast ORF deletion collection, mother cells typically arrest in an unbudded state about two-thirds of the time; however, we have observed several mutations that significantly alter the percentage of budded yeast at the end of life. Here, we present an initial report of these observations containing terminal morphology data for more than 5000 individual *S. cerevisiae* mother cells. These data indicate that, within the wild-type population, mother cells that terminally arrest in a budded state tend to have shorter RLS than cells that arrest in the unbudded state. This is most notable in the diploid population where budded cell cycle arrest contributes substantially to individual variation with respect to RLS. We expand upon prior evidence that a budded terminal morphology is associated with genomic instability by showing that cells lacking DNA-repair enzymes arrest with a significantly higher percentage of budded cells, as do cells lacking the sirtuin histone deacetylase enzyme Sir2.

Table 1. Summary of prior studies reporting terminal budding morphology of wild-type yeast strains

Strain background	Percent budded	Percent unbudded	*N*	Reference
W303	~60	~40	50	McVey *et al.* (2001)
W303	~55	~45	~50	Merker & Klein (2002)
W303	~55	~45	~40	Falcon & Aris (2003)
KT308	47	53	~50	Pichova *et al.* (1997)
JC482	71	29	~50	Pichova *et al.* (1997)

Materials and methods

Replicative life spans

All mutant strains examined were derived from the MATα ORF deletion collection and are isogenic to the parental BY4742 strain (Brachmann *et al.*, 1998; Winzeler *et al.*, 1999) or were derived from direct transformation of BY4742 with a PCR-generated disruption cassette. Strain genotypes were verified by PCR genotyping. The pMoBY strain contains the vector CEN-marked plasmid and a dubious gene that does not code for a functional protein, YGR045C, and acts as a vector control. Yeast RLS assays were performed as previously described (Kaeberlein *et al.*, 2004; Steffen *et al.*, 2009). In short, virgin daughter cells were isolated from each strain and then allowed to grow into mother cells while their corresponding daughters were microdissected and counted until the mother cell could no longer divide. YEP agar plates (1% yeast extract, 2% bacto-peptone, 2% agar) containing 2% glucose were utilized and strains were grown at 30 °C except for dietary restriction experiments where the glucose was kept at 0.05%. Daughter cells were removed from each mother cell each 1–2 h by micromanipulation. Terminal morphology was defined as the budded state of the mother cells upon senescence. Cells were scored as senescent when they had failed to divide for at least 8 h of incubation at 30 °C. The data shown here were pooled across multiple experiments and were generally obtained in 20 cell sets. All experiments were performed by a team of dissectors working in shifts who were blinded to the identity of the strains under examination in any given experiment. Statistical significance for differences in median life span was determined using the Wilcoxon's rank-sum test. Budded and unbudded states were determined visually for each mother cell assayed and statistical comparisons of budding rates utilized Fischer's exact two-tailed test.

Results

Unbudded senescence correlates with longer life span in isogenic wild-type yeast

End-of-life terminal morphology was recorded and analyzed for wild-type haploid *MAT***a** (BY4741, 1792 cells),

Table 2. End-of-life budding inversely correlates with life span. Quartiles of the shortest and longest lived 25% of isogenic wild-type cells are compared to the complete set of data. All *P* values were calculated by a two-tailed Fischer's exact test and compare the percent budded rates

	RLS	Un-budded	Budded	%U	U RLS	U CV	%B	B RLS	B CV	U RLS vs. B RLS *P*
BY4741 (*mat a*)	26.6	1334	454	74.6	27.2	0.37	25.4	25.0	0.36	2.1E-37
BY4742 (*mat* α)	25.9	2566	1227	67.7	26.6	0.38	32.3	24.5	0.35	2.0E-171
BY4743 (diploid)	33.7	415	114	78.4	35.6	0.32	21.6	26.6	0.42	2.1E-13

RLS, replicative lifespan; U, unbudded; B, budded; CV, coefficient of variation.

wild-type haploid *MAT*α (BY4742, 3794 cells), and wild-type diploid (BY4743, 529 cells) yeast subjected to RLS analysis under standard conditions (Steffen *et al.*, 2009). In all three backgrounds, a majority of cells arrested in a budded state (Table 2). Likewise, in each case, the cells that arrested in a budded state lived shorter on average than those that arrested in an unbudded state (Fig. 1). The difference in longevity of cells that arrest in an unbudded state vs. a budded state is particularly apparent in the diploid cell population, where cells showing a budded terminal morphology had an average RLS of 26.6 compared to 35.6 for cells that arrest in the unbudded state. Interestingly, the coefficient of variance was larger for diploid cells that arrest in a budded state (0.42) relative to cells that arrest in an unbudded state (0.32), indicating that this premature cell cycle arrest contributes to individual variation in life span within this population.

A prior study found that plasmid accumulation can reduce RLS in the W303AR5 strain background and, at least in some cases, alter the terminal morphology distribution in this strain (Falcon & Aris, 2003). We have recently been using the pMoBY CEN-marked plasmid collection (Ho *et al.*, 2009) to perform overexpression studies for RLS. Consistent with the results of Falcon and Aris (Falcon & Aris, 2003), we find that BY4742 wild-type cells containing a CEN-marked pMoBY vector are slightly short-lived relative to untransformed cells and also tend to arrest more frequently as budded cells (Table 3). As in the prior cases, the subset of plasmid-containing cells arresting in the budded state are shorter-lived than those that arrest in the unbudded state.

Life span extension is not always associated with altered terminal morphology

As the longest-lived wild-type cells arrest in the unbudded state, we hypothesized that mutations that extend wild-type RLS might also cause more cells to arrest in the unbudded state. To test this hypothesis, we examined the terminal morphologies of long-lived mutants for which we had terminal morphology data for at least 60 mother cells. Gpr1, Tor1, and Sch9 are important nutrient sensors that have been previously implicated in mediating increased RLS in response to dietary restriction, and

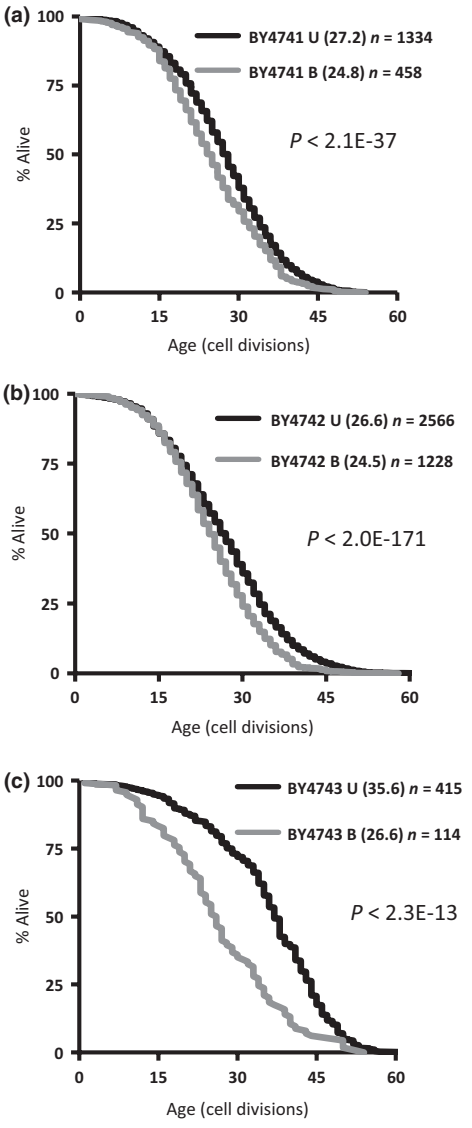

Fig. 1. End-of-life budded cells terminally arrest earlier than unbudded cells. Replicative life span curves for combined experiments containing wild-type BY4741 (MATa), BY4742 (MATα), and BY4743 (diploid) are shown for cells that arrested in an unbudded (U) or budded (B) state.

deletion of the genes coding for any one of these proteins is sufficient to increase life span (Lin *et al.*, 2000; Fabrizio *et al.*, 2004; Kaeberlein *et al.*, 2004, 2005a, b). In the case

Table 3. Mutations that alter RLS also alter end-of-life budding rates. Cells that arrest in a budded state tend to live shorter than unbudded cells and have a higher coefficient of variation (CV)

	RLS	Unbudded	Budded	%U	U RLS	U CV	%B	B RLS	B CV	%Budded vs. BY4742 *P*	U vs. B RLS *P*
BY4742	25.9	2566	1228	67.6	26.6	0.38	32.4	24.5	0.35		2.0E-171
gpr1Δ	37.6	69	11	86.3	39.0	0.23	13.8	28.9	0.53	< 0.0002	3.2E-02
sch9Δ	34.0	186	34	84.5	34.8	0.34	15.5	29.6	0.51	< 0.0001	4.5E-02
rpl22aΔ	35.2	74	24	75.5	37.4	0.41	24.5	28.6	0.51	0.125	1.9E-02
tor1Δ	29.8	511	227	69.2	30.5	0.38	30.8	28.3	0.36	0.389	7.8E-03
tif1Δ	26.0	54	26	67.5	27.1	0.36	32.5	23.6	0.42	1.000	1.3E-01
rpl20bΔ	35.3	453	224	66.9	36.7	0.31	33.1	32.5	0.41	0.722	6.8E-05
sps1Δ	28.0	30	50	37.5	29.4	0.34	62.5	27.1	0.29	< 0.0001	6.9E-01
idh2Δ	31.3	102	78	56.7	33.4	0.33	43.3	28.6	0.36	0.003	6.4E-03
rad53Δ	5.1	42	92	31.3	5.9	0.74	68.7	4.7	0.79	< 0.0001	1.0E-01
rad52Δ	11.9	52	128	28.9	13.3	0.69	71.1	11.3	0.67	< 0.0001	1.6E-01
rad51Δ	11.4	33	87	27.5	14.4	0.65	72.5	10.3	0.69	< 0.0001	2.9E-02
rad50Δ	8.1	11	28	28.2	8.8	0.68	71.8	7.8	0.75	< 0.0001	6.7E-01
pMoBY	19.4	107	90	54.3	19.9	0.42	45.7	18.9	0.47	0.0002	5.3E-01
sir2Δ	13.6	169	464	26.7	14.6	0.38	73.3	13.3	0.40	< 0.0001	9.9E-03
SIR2OX	34.0	95	25	79.2	36.1	0.34	20.8	26.2	0.44	0.007	3.2E-04
fob1Δ	29.4	375	103	78.5	30.9	0.36	21.5	23.9	0.50	< 0.0001	4.1E-08
sir2Δ fob1Δ	24.7	361	347	51.0	26.6	0.43	49.0	22.8	0.47	< 0.0001	1.7E-05
sir2Δ fob1Δ (0.05%D)	34.6	146	113	56.4	37.5	0.42	43.6	30.8	0.47	< 0.0003	3.6E-04

of *gpr1Δ* and *sch9Δ* cells, the percentage of cells arresting in the unbudded state were significantly increased relative to wild-type BY4742 cells (Table 3). In the case of *tor1Δ* cells, there was a trend toward a higher percentage of unbudded terminal cells that did not reach statistical significance.

The association between increased unbudded terminal morphology and longevity was not apparent in all long-lived mutants, however. For example, deletion of the isocitrate dehydrogenase gene *IDH2* or the meiotic gene *SPS1* also increases RLS (Kaeberlein *et al.*, 2005a, b; Managbanag *et al.*, 2008). Yet, *idh2Δ* and *sps1Δ* cells showed a significant decrease in the percent of cells with unbudded terminal morphology (Table 3). Unlike the other long-lived mutants tested, both *idh2Δ* and *sps1Δ* cells are also respiratory deficient (Merz & Westermann, 2009), but whether this defect is related to the terminal morphology phenotype remains unclear. In every genotype examined, however, the cells that arrested in the unbudded state trended toward longer RLS than those that arrested in the budded state (Table 3), and in most genotypes, unbudded cells lived significantly longer (Fig. 2).

Ribosomal DNA stability and terminal morphology

The ribosomal DNA (rDNA) has been repeatedly shown to be an important locus determining replicative longevity of yeast cells (Sinclair & Guarente, 1997; Ganley *et al.*, 2009; Lindstrom *et al.*, 2011). The yeast rDNA exists as a tandem repeat of 50–200 copies of a 9.1-kb sequence (Petes & Botstein, 1977; Rustchenko & Sherman, 1994). This repeated structure leads to elevated rates of homologous recombination within the rDNA. The replication fork block protein Fob1 has been previously shown to limit RLS by promoting rDNA instability and the resulting formation of extrachromosomal rDNA circles (Defossez *et al.*, 1999). The sirtuin histone deacetylase Sir2 antagonizes Fob1 by promoting enhanced rDNA stability and reduced formation of extrachromosomal rDNA circles (Kaeberlein *et al.*, 1999). Deletion of *FOB1* extends RLS while deletion of *SIR2* shortens it. Correspondingly, deletion of *FOB1* caused a greater proportion of cells to terminally arrest in the unbudded state, and deletion of *SIR2* caused more cells to terminally arrest in the budded state (Table 3).

Deletion of *FOB1 in sir2Δ* cells restores RLS to the level of wild-type mother cells (Kaeberlein *et al.*, 1999) and enhances the ability of dietary restriction to increase RLS (Kaeberlein *et al.*, 2004; Delaney *et al.*, 2011). Unexpectedly, *sir2Δ fob1Δ* cells under both control and dietary-restricted conditions arrested in a budded state more frequently than wild-type cells (Table 3). Thus, deletion of *FOB1* only partially suppresses the terminal morphology defect of *sir2Δ* cells and DR has no significant effect on terminal morphology in *sir2Δ fob1Δ* cells, despite the large increase in RLS. In the BY4742 wild-type background, DR does not yield as great a life span increase and no significant change in terminal morphology is observed.

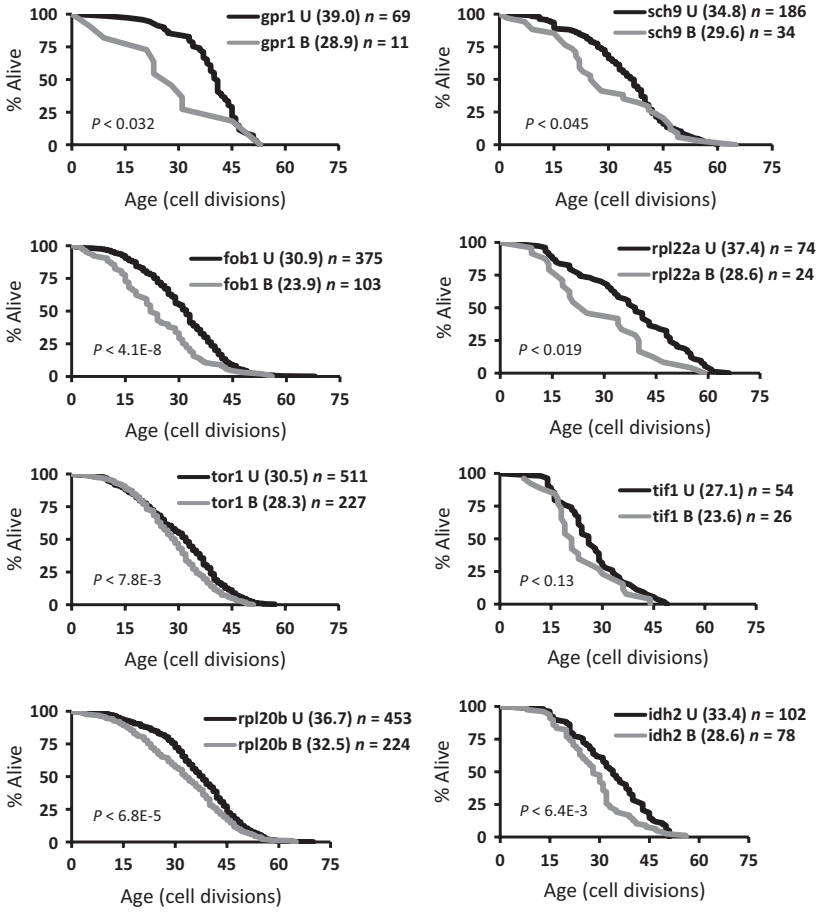

Fig. 2. Within a long-lived mutant strain, cells that terminally arrest in a budded state are shorter-lived. Replicative lifespan curves for long-lived mutants are shown for cells that arrested in an unbudded (U) or budded (B) state.

DNA damage repair mutants have increased budded terminal morphology

As two prior studies had shown an increased proportion of senescent budded cells in mutants with reduced genomic stability (McVey *et al.*, 2001; Merker & Klein, 2002), we tested whether DNA repair-deficient mutants would also show altered terminal morphologies. For this experiment, we examined four highly conserved DNA-repair genes: *RAD50*, *RAD51*, *RAD52*, and *RAD53*. Rad53 acts downstream of the Mec1 kinase to signal for G1 arrest and does not directly contribute to DNA damage repair (Sidorova & Breeden, 1997; Ma *et al.*, 2006). Rad50 is part of the Mre11 complex that acts in multiple DNA repair and G1 arrest signaling pathways, including homologous recombination, sensing of double-stranded breaks, and suppression of chromosomal rearrangements [reviewed in (Symington, 2002; Krogh & Symington, 2004)]. Rad51 and Rad52 are both directly involved in the repair of DNA double-stranded breaks (New *et al.*, 1998; Shinohara & Ogawa, 1998; Krogh & Symington, 2004). In each case, the gene deletion reduced RLS (Fig. 3), and cells arrested with a budded morphology > 70% of the time (Table 3). Even in these cases of decreased life span, the trend toward increased RLS among those mother cells that arrested in an unbudded state was preserved, although it did not achieve statistical significance on an individual strain basis.

Cells that arrest in a budded state can be further subclassified based on whether their final arrest occurs with a small bud, a large (or symmetric) bud, or multiple daughter and/or granddaughter cells (clustered) that cannot be separated by micromanipulation (Fig. 3b). The subtypes of budded arrest also differ in *rad*Δ cells compared to wild type (Fig. 3c). The percentage of cells arresting in the budded clustered and large-budded morphological categories was significantly increased in all *rad*Δ mutants examined.

Discussion

The stochastic nature of aging is evident in yeast from the variation in both longevity and terminal morphology of genetically identical mother cells maintained under essentially identical conditions. Here, we have demonstrated a novel interaction between longevity and terminal

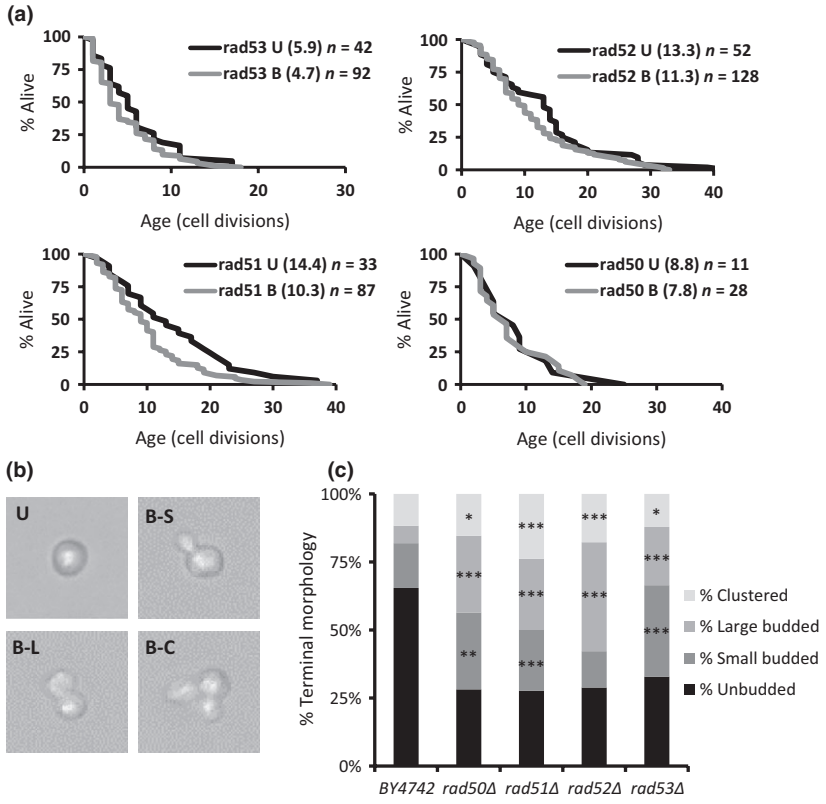

Fig. 3. (a) DNA-damage mutants increase terminal rates of budding and decrease RLS. Replicative life span curves for *rad50Δ*, *rad51Δ*, *rad52Δ*, or *rad53Δ* mutants are shown for cells that terminally arrested in an unbudded (U) or budded (B) state. Comparable wild-type cells are shown in Fig. 1. (b) Examples of unbudded, small-budded, large-budded, and clustered cells are shown. (c) Mutants in selected RAD genes increase relative amounts of large-budded and clustered terminal cell morphologies. Asterisks denote significant increases compared to BY4742; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

morphology in wild-type cells, by showing that, within the same population, mother cells arresting in the budded state tend to be shorter-lived than mother cells arresting in the unbudded state. This was true in both haploid backgrounds, as well as the diploid background of the yeast deletion collection. This relationship also held true across several mutant backgrounds, showing altered distributions of terminal morphologies. In some cases, the instability that gives rise to higher terminal budding rates may be limiting for life span, because we observed an inverse correlation between strain end-of-life budding rates and overall mean life span (Fig. 4). Taken together, these observations suggest that within a population of yeast cells, some individuals will succumb to a life span-limiting event that corresponds with budded cell cycle arrest, while others will escape this fate and achieve their full replicative potential.

The relationship between stochastic aging events and terminal morphology is most evident in the diploid BY4743 population. The coefficient of variation for individual cell life span was dramatically reduced in the unbudded cell population, relative to cells that arrested in the budded state. A similar relationship was not detected in either haploid background, perhaps due to the smaller difference in absolute life span between the budded and unbudded cell sets; however, when the results from all

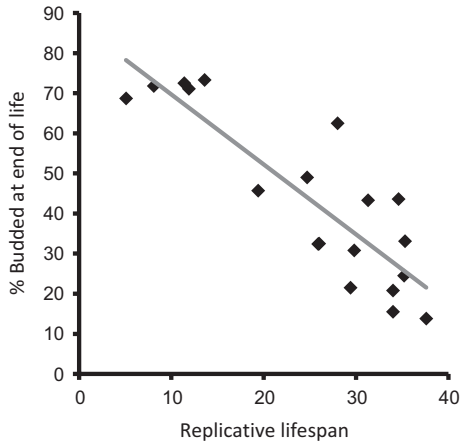

Fig. 4. Correlation plot of end-of-life budding rates to mean life span, from the strains in Table 2. A significant inverse relationship is observed ($R^2 = 0.7419$, regression $P < 2.2E\text{-}6$).

different genotypes were examined, those cells that arrested in a budded state tended to have a higher coefficient of variance in their life span compared to unbudded cells (Fig. 5). This higher variation is consistent with the interpretation that terminal buds result from stochastically arising damage in the mother cell that contributes to individual variation in longevity.

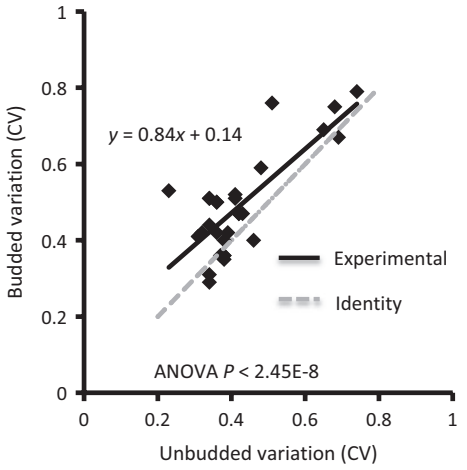

Fig. 5. The coefficient of variation (CV) of cells that terminally arrested in the budded state tends to be higher than that of cells arresting in an unbudded state. The data points represent the CV of budded cell life span compared to the CV of the experimentally identical isogenic population that arrested in an unbudded state. The identity line is shown as a representation of where the trendline should lie if there were no difference in CV between budded arrest and unbudded arrest populations. The ANOVA statistic is compared against this identity line.

What might be the nature of the event(s) resulting in premature arrest in the budded state? Prior data have suggested that genomic instability at the rDNA is associated with a higher percentage of mother cells arresting in a budded state (McVey *et al.*, 2001; Merker & Klein, 2002), and our data are consistent with this observation. Genomic instability or defects in DNA repair outside of the rDNA also appear to be associated with a higher proportion of cells terminally arresting in the budded state, as evidenced from the terminal morphologies of *rad50Δ*, *rad51Δ*, *rad52Δ*, and *rad53Δ* cells. It is interesting to note that *rad52Δ* cells, in particular, have very low levels of rDNA recombination (Park *et al.*, 1999), suggesting that the aging-related damage that results in a non-G1 arrest need not be restricted to the rDNA, although the rDNA may be the primary site of such damage in otherwise wild-type cells. During chronological aging, DRor deletion of the gene encoding the S6 kinase ortholog *SCH9* decrease the percent of cells that arrest with buds, and this is associated with less replication stress (Weinberger *et al.*, 2010). We have similarly found a reduction in the percent of budded under conditions that extend chronological life span as well as subsequent RLS (Murakami *et al.*, 2012).

It is also of interest that *sir2*Δ mutant cells phenocopy the DNA damage–sensitive *rad*Δ cells with respect to terminal morphology, yet deletion of *FOB1* only partially alleviates this phenotype, despite restoring life span and rDNA recombination rates to wild-type levels. In addition to destabilizing the rDNA, deletion of *SIR2* also results in loss of silencing near telomeres, and recent studies have suggested that this function of Sir2 may be particularly important for ensuring replicative longevity (Dang *et al.*, 2009; Chan *et al.*, 2011). Thus, it may be that the increase in terminal budding in cells lacking Sir2 occurs in response to a DNA-damage signal originating from destabilized telomeres.

The observation that some long-lived mutants have a higher percentage of cells arrest in the unbudded state suggests that one path to longer life span may be achieved by reducing the proportion of cells dying from premature cell cycle arrest outside of G1. This is clearly not the only way to achieve life span extension, however, because both *idh2Δ* and *sps1Δ* cells are long-lived while also having a greater proportion of cells terminally arrest with buds. An even more striking example of this is the case of *sir2Δ fob1Δ* cells subjected to dietary restriction. These mother cells live, on average, about 50% longer than wild-type cells, but have a greater proportion of cells terminally arrest in the budded state. Although many strains in the yeast ORF deletion collection are known to carry secondary mutations (Cheng *et al.*, 2008; Steffen *et al.*, 2012), we do not believe that secondary mutations account for the observed terminal morphology distributions in the mutants described here, because we have independently rederived a majority of the strains examined in this study.

At least some aspects of the genetic control of longevity in yeast are conserved in multicellular eukaryotes (Smith *et al.*, 2008; Fontana *et al.*, 2010; Longo *et al.*, 2012). We speculate that the mechanisms underlying control of terminal morphology in yeast, as well as their stochastic nature, may also be relevant in mammalian cells. The ATR tumor suppressor pathway acts to prevent ectopic entry into S phase. Mec1 is the ATR homologue in yeast (Cimprich *et al.*, 1996), and it activates Rad53 to induce cell cycle arrest (Sweeney *et al.*, 2005; Ma *et al.*, 2006), in particular due to MMS-induced DNA damage (Sidorova & Breeden, 1997). Rad53 has been implicated in modulating appropriate entry into S phase in unstressed conditions as well (Sidorova & Breeden, 2002). The finding that *rad53*Δ mutant cells have a high proportion of cells that arrest in the budded state supports the idea that this regulation is important during aging. Thus, yeast RLS may provide a useful method for researching the mechanisms of cell cycle arrest in a model system without the confounding effects of background mutations found in immortal cell lines.

There is little doubt that apparently stochastic events occurring during aging play an important role in determining individual life span (Kirkwood *et al.*, 2005). In yeast, one form of this stochasticity arises from damage

that causes some cells to arrest prematurely, an event that can be detected by a budded terminal morphology. This stochasticity can be modulated genetically, but in all cases examined was never completely removed. Although the precise molecular nature of the damage leading to non-G1 arrest during yeast aging is unclear, one potential contributing cause is DNA damage or genomic instability. There is abundant evidence that elevated levels of DNA damage and genomic instability induce early cell death and progeroid phenotypes in mammals (Burtner & Kennedy, 2010; Kennedy *et al.*, 2012). It seems plausible that stochastic events occurring during normal aging cause some cells in mammals to die prematurely, as well, perhaps akin to the subpopulation of wild-type yeast that arrest in a budded state. Thus, it may be that by understanding the genetic and environmental factors that modulate terminal morphology of yeast mother cells, we will gain insight into the mechanisms that govern stochastic aspects of aging both in yeast and in higher eukaryotes.

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Authors' contributions

J.D. and M.K. jointly conceived this study. B.O. contributed to large-scale data analysis. All except B.O., B.K.K. and M.K. performed experiments. J.D., A.C., B.K.K. and M.K. wrote the manuscript.

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