A Functional Analysis Reveals Dependence on the Anaphase-Promoting Complex for Prolonged Life Span in Yeast

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ABSTRACT

Defects in anaphase-promoting complex (APC) activity, which regulates mitotic progression and chromatin assembly, results in genomic instability, a hallmark of premature aging and cancer. We investigated whether APC-dependent genomic stability affects aging and life span in yeast. Utilizing replicative and chronological aging assays, the APC was shown to promote longevity. Multicopy expression of genes encoding Snf1p (MIG1) and PKA (PDE2) aging-pathway components suppressed apc5Δ phenotypes, suggesting their involvement in APC-dependent longevity. While it is known that PKA inhibits APC activity and reduces life span, a link between the Snf1p-inhibited Mig1p transcriptional modulator and the APC is novel. Our mutant analysis supports a model in which Snf1p promotes extended life span by inhibiting the negative influence of Mig1p on the APC. Consistent with this, we found that increased MIG1 expression reduced replicative life span, whereas mig1Δ mutations suppressed the apc5Δ chronic aging defect. Furthermore, Mig1p and Mig2p activate APC gene transcription, particularly on glycerol, and mig2Δ, but not mig1Δ, confers a prolonged replicative life span in both APC5 and apc5Δ cells. However, glucose repression of APC genes was Mig1p and Mig2p independent, indicating the presence of an uncharacterized factor. Therefore, we propose that APC-dependent genomic stability is linked to prolonged longevity by the antagonistic regulation of the PKA and Snf1p pathways.

The anaphase-promoting complex (APC), an evolutionarily conserved, large multi-protein complex that is essential for yeast viability, functions as a ubiquitin-protein ligase (E3; Zachariae and Nasmyth 1999; Harper et al. 2002). The APC controls progression through mitosis by targeting mitotic inhibitors for degradation and this activity is regulated at the protein level by a complex network of interactions (Kotani et al. 1998; Rudner and Murray 2000). One notable example of APC regulation is the negative influence of RAS/protein kinase A (PKA) signaling on the yeast APC (Yamashita et al. 1996; Kotani et al. 1998; Irniger et al. 2000; Bolte et al. 2003). PKA phosphorylates the APC subunits Cdc27p and Apc1p, which is sufficient to inactivate the APC (Kotani et al. 1998). PKA signaling is considered to play a role in maintaining chromosomal stability (Matyakhina et al. 2002). Defects that alter APC activity are associated with cancer development in humans (Pray et al. 2002; Liu et al. 2003; Wang et al. 2003; Song et al. 2004). Recently, we isolated and described a mutation in the Apc5p APC subunit that rendered cells temperature sensitive (ts) at 37°C, predisposed to chromosome loss, and chromatin assembly defective in vitro (Harkness et al. 2002, 2003). These observations suggest that the APC is also critical for chromosome maintenance, chromatin metabolism, and genomic stability during mitosis.

Recent studies have demonstrated the striking involvement of chromatin in the aging process (reviewed in Campisi 2000; Chang and Min 2002; Hekimi and Guarente 2003). Chromatin is a complex molecular structure composed of nucleosomal repeats in which 147 bp of DNA are wrapped around two copies of the four core histones, H2A, H2B, H3, and H4 (Luger et al. 1997; Richmond and Davey 2003). The critical components within chromatin that are central to the control of many cellular processes are the histones. Chromatin-associated histones are post-translationally modified by a variety of activities and these modifications are responsible for the control of a vast number of cellular activities (van Leeuwen and Gottschling 2002; Fischle et al. 2003). Deacetylation of lysine residues within histone N-terminal tails is believed to play a large role in gene silencing by creating conformational changes within chromatin that render it resistant to transcription factors (Grunstein 1997; Eberharter and Becker 2002). Silencing of the yeast rDNA locus, in particular, represses rDNA recombination, reducing the formation of extrachromosomal rDNA circles (ERCs), and this is believed to enhance replicative life span, a measure of how many daughter cells a given mother cell can produce (Kennedy et al. 1994; Sinclair and Guarente 1997; Defossez et al. 2001). The repression of ERC formation is believed to be at the heart of the enhanced

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A series of experiments have shown that conserved glucose-signaling pathways from yeast to humans shorten life span (reviewed in Longo and Fabrizio 2002). PKA signaling, which responds to elevated glucose levels, has been shown to shorten both replicative and chronological life span in yeast (Sun et al. 1994; Lin et al. 2000; Fabrizio et al. 2001). Opposed to the replicative aging assay, which measures the life span of dividing cells, the chronological life span assay determines the life span of nondividing cells (Fabrizio et al. 2001). Since glucose activates PKA signaling, PKA mutants have been used to mimic caloric restriction (Lin et al. 2000). Hence, the downregulation of PKA was shown to mimic the effects of caloric restriction on replicative life span. Therefore, life span in both dividing and nondividing yeast cells appears to be regulated in a similar fashion by the glucose/PKA signaling pathway.

Histone modifications other than acetylation are also known to influence aging in yeast. For example, the Snf1p kinase in yeast has been shown to phosphorylate histone H3 on Ser10 and the effects of this modification are desilencing and increased recombination at the rDNA locus, leading to ERC formation and decreased replicative life span (Lin et al. 2003). Opposed to PKA, Snf1p is inhibited by glucose (Jiang and Carlson 1996; reviewed in Johnston 1999). The role Snf1p plays in yeast replicative aging appears to be complex. Both disruption and forced expression of SNF1 have been shown to lead to a reduced life span (Ashrafi et al. 2000; Lin et al. 2003). These studies indicate that Snf1p activity must be tightly regulated as both increased and decreased Snf1p activity shortens life span. Nonetheless, as Snf1p is required for transcriptional activation upon glucose limitation (Carlson 1999), Snf1p walks a fine line between gene expression and desilencing, with accelerated aging hanging in the balance.

Snf1p is the yeast homolog of the human AMP kinase and both are involved in regulating cellular stress responses (Hardie et al. 1998). Snf1p is the key component of a heterotrimeric kinase complex containing a catalytic α-subunit (Snf1p), an activating γ-subunit (Snf4p) and one of three β-subunits (Sip1p, Sip2p, or Gal83p; Schmidt and McCartney 2000; Vincent et al. 2001; Nath et al. 2002). The β-subunits have been shown to direct the Snf1 complex to different subcellular compartments upon glucose limitation (Vincent et al. 2001). For example, Sip1p is believed to direct Snf1p to the vacuole, Sip2p retains Snf1p in the cytosol, and Gal83p directs Snf1p into the nucleus. In the presence of high glucose, inhibition of Snf1p occurs by the binding of the Snf1p kinase catalytic domain to the Snf1p autoinhibitory domain, thereby forming an inactive complex (Jiang and Carlson 1996; reviewed in Johnston 1999). When glucose becomes limiting, two events that independently lead to Snf1p activation are believed to occur: (i) Snf1p is potentially phosphorylated by several kinases, Pak1p, Tos3p, and Elm1p (Hong et al. 2003; Nath et al. 2003), and (ii) Snf4p binds to the autoinhibitory domain in Snf1p, displacing and freeing the Snf1p kinase domain (Jiang and Carlson 1996; McCartney and Schmidt 2001). Once active, Snf1p phosphorylates and inhibits its targets, most notably the transcriptional repressor Mig1p, which then allows the cell to transcribe genes required for the utilization of alternate carbon sources (Carlson 1999).

Mig1p is a major Snf1p target in the nucleus that represses transcription from glucose-repressible promoters (Nehlin and Ronne 1990; Treitel et al. 1998). Mig1p contains zinc-finger motifs related to those found in the Wilms tumor suppressor protein that are likely involved in making specific promoter DNA contacts (Nehlin and Ronne 1990; Leon and Roth 2000). The yeast genome encodes additional proteins that share these motifs (Bohm et al. 1997), some of which are functionally redundant with Mig1p (Lutfiyya and Johnston 1996; Lutfiyya et al. 1998). A notable example is Mig2p, which, together with Mig1p, represses the transcription of many genes in the presence of glucose. However, Mig2p activity does not appear to be Snf1p or glucose dependent (Lutfiyya et al. 1998). Mig1p modulates the transcription of glucose-repressible genes by binding to specific promoter sequences, also recognized by Mig2p, and then recruiting specific transcriptional repressors (Treitel and Carlson 1995; Lutfiyya et al. 1998). Upon glucose limitation, Snf1p phosphorylates Mig1p, leading to export of Mig1p from the nucleus and allowing derepression of genes required to metabolize alternative carbon sources (Ostling and Ronne 1998; Treitel et al. 1998; DeVit and Johnston 1999; Smith et al. 1999).

This report describes the identification and characterization of a signaling pathway that influences yeast longevity by regulating the activity of the cell-cycle monitor, the APC (Zachariae and Nasmyth 1999; Harper et al. 2002). The results and extended studies initiated from a multicopy suppressor screen of the apc5Δ ts phenotype are presented here. The recovery of MIG1 and PDE2 (Pde2p is involved in downregulating PKA activity) as multicopy suppressors of apc5Δ phenotypes suggests that the APC is a central player in yeast aging and serves as the focal point for life-span regulation by multiple signaling pathways. Here, APC mutants are shown to senesce prematurely in both the replicative and chronological aging assays. We conclude that the Snf1p kinase pathway positively influences APC activity by alleviating the negative effects of Mig1p on APC gene expression.
Yeast strains and plasmids: Table 1 lists the yeast strains used in this study. All strains were S288c derivatives unless stated otherwise. Standard genetic techniques were performed as described to generate the strains listed (Guthrie and Fink 1991). All other strains were obtained from the sources shown. The CEN-APC5 plasmid, pH101, was described previously (Harkness et al. 2002). Mig1p was cloned from a plasmid insert isolated from a 2μ aple5-Δ to suppression screen (see Materials and Methods).

and subsequent life-span reduction. APC4 and APC9 transcription is glucose repressed, but this does not require Mig1p or Mig2p. Rather, Mig1p and Mig2p act as activators at APC4 and APC9 promoters. Mig2p, however, plays a larger role than Mig1p in life-span reduction. Overall, the results presented here suggest that APC activity is critical for enhanced replicative and chronological life span.

### Materials and Methods

**Yeast strains and plasmids:** Table 1 lists the yeast strains used in this study. All strains were S288c derivatives unless stated otherwise. Standard genetic techniques were performed as described to generate the strains listed (Guthrie and Fink 1991). All other strains were obtained from the sources shown. The CEN-APC5 plasmid, pH101, was described previously (Harkness et al. 2002). Mig1p was cloned from a plasmid insert isolated from a 2μ aple5-Δ to suppression screen (see Materials and Methods).
below). A plasmid expressing 2µ-MIG1, pJO114, was obtained from H. Ronne. A plasmid expressing CEN-MIG1-GFP was obtained from M. Johnston. The 2µ-GST-APC5 and 2µ-GST-APC10 plasmids were isolated from the Research Genetics (ResGen, Huntsville, AL) Yeast ExClones library (MARTZEN et al. 1999) and confirmed by plasmid rescue and Western blotting with antibody against the GST epitope. The CEN-SNFI-GFP (pOV84) and CEN-SNF4-GFP (pOV76) plasmids were generous gifts from M. Carlson.

**Media and methods:** Media were prepared according to AUSUBEL et al. (1995). Glucose was added to a final concentration of 3% when apc5CA cells were assayed at 37°C. Otherwise, glucose, galactose and glycerol were used at a final concentration of 2%. *Escherichia coli* strains JM109 and DH10B were used to propagate DNA plasmids. DNA manipulations such as restriction enzyme digests, DNA minipreps, yeast and *E. coli* transformations, and yeast genomic DNA preparation were carried out according to standard protocols (AUSUBEL et al. 1995). *In vitro* chromatin assembly assays have been described previously (HARKNESS et al. 2002, 2003). Spot dilution assays were conducted by pipetting 5 µl of cells from samples generated from a 10-fold dilution series onto the various media and grown at the temperatures indicated. The starting spot generally contained 5 × 10⁶ cells.

**Multicopy suppressor screen:** To isolate genes that suppressed the apc5CA phenotype when expressed in multicopy, a 2µ-based plasmid library (NASMYTH and REED 1980; ATCC no. 37323) was transformed into apc5CA rm2 cells (YTH249). All 2000 transformants that grew at 30°C were replica plated to 37°C. Five colonies continued to grow, and plasmids were recovered, retested, and sequenced. Three separate inserts were represented within the five plasmids. Subcloning revealed that the genes MIG1 and PDE2 were responsible for the suppression. All inserts were subsequently tested in apc5CA single mutants and shown to suppress the apc5CA phenotype.

**Life-span determination:** Replicative, or generational, life span of the strains tested in this study was based on previously published protocols (KENNEDY et al. 1994). Briefly, diluted cells from a fresh culture were struck onto fresh YPD (1% yeast extract; 2% peptone; 2% glucose) plates and grown overnight at 30°C. Drop-out media was used if plasmids were to be maintained. The next day ~30–50 cells containing small buds were micromanipulated to isolated areas of the plate. The small daughter buds were kept as the starting mother cells. All additional buds from the starting mother cells were scored and discarded. The plates were kept at room temperature or 30°C during working hours and stored at 4°C or 16°C overnight.

Chromosomal life-span experiments were based on previously published methods (FARRIZIO et al. 2001). Cells were inoculated into 10 ml of fresh complete media (CM; 0.17% yeast nitrogen base minus amino acids; 0.5% (NH₄)₂SO₄; 2% glucose; all required amino acids) and grown for 2 days to reach stationary phase. All cells used in this study were assayed initially in CM. Alternatively, once the cells reached stationary phase in CM, the cells were harvested, washed two times with H₂O, and then resuspended in 10 ml H₂O. Cells that senesced prematurely in CM also senesced prematurely in H₂O. However, in some cases, the cells exhibited a recovery phase in CM that was most likely due to the uptake of nutrients released from dead cells. This recovery was not observed when the cells were maintained in H₂O. Cell viability was determined by plating a known number of cells on YPD plates. Cell viability counts were started on the third day of growth, which constituted day 1 in our experiments. On the days shown, cells were harvested and resuspended in 10 ml of fresh H₂O and cell viability was determined. Percentage of viability was calculated by dividing the viable cells from each day by the viable cells on day 1. Experiments were repeated at least three times.

**Northern analysis:** A Northern analysis of yeast RNA was used to determine the relative abundance of each gene under repressed (4% glucose) and derepressed (5% glycerol, 0.1% glucose) conditions. Strains expressing plasmids were grown under repressing conditions (SD-leu, 4% glucose; synthetic defined: 0.17% yeast nitrogen base minus amino acids, 0.5% (NH₄)₂SO₄, 4% glucose, and all required amino acids except leucine). Probes were generated by PCR of genomic DNA using primers amplifying from the start to stop sites of each gene. The PCR fragments were cloned into the pCR4-TOPO vector. The inserts were then released by digestion with EcoRI, gel purified, and random labeled with [α-³²P]CTP. This analysis showed that the APC gene transcripts were exceedingly rare. Thus, special hybridization techniques were employed to increase the target signal. Briefly, an amplification buffer (Ambion, Austin, TX) was utilized with overnight hybridization at 42°C. Yeast genes with a more abundant message (i.e., SUC2 and RDN1) were subjected to a standard 68°C hybridization protocol (Current Protocols) as previously described (HARKNESS et al. 2002). Amplified, rare APC messages were subjected to ~80° autoradiography with intensifying screens (Kodak) for up to a week while more abundant signals required only hours to overnight exposures.

**RESULTS**

The APC is required for longevity: The APC is a large multisubunit complex required for the degradation of mitotic inhibitors and cell-cycle progression. On the basis of the observed connection between genomic stability and the APC (HARTWELL and SMITH 1985; PALMER et al. 1990; HARKNESS et al. 2002; LIU et al. 2003; SHIN et al. 2003) and the link between genomic instability and cancer (reviewed in CHARAMES and BAPAT 2003; STORCHHOVA and PELLMAN 2004), we predicted that APC mutants would age rapidly. We used two assays to investigate whether the APC influenced aging: replicative and chronological life span. Replicative life span in yeast is determined by scoring the number of daughters a single mother cell produces prior to senescence (KENNEDY et al. 1994). Chronological aging in yeast is measured by following how long a population of yeast cells remains viable after reaching stationary phase (FARRIZIO et al. 2001). Thus, by using the two assays, it is possible to measure aging in dividing and nondividing cells.

We chose to measure replicative and chronological life span in apc5CA cells, as apc5CA cells suffer genomic instability (HARKNESS et al. 2002). Cells expressing apc5CA generated fewer daughters than isogenic wild-type cells (Figure 1A) and senesced faster (Figure 1B) than isogenic wild-type cells after reaching stationary phase, indicating that the APC is indeed required for normal life span. However, Apc5 was recently found in complexes other than the APC in humans (KOLOTEVA-LEVINE et al. 2004). Therefore, to determine whether the apc5CA-accelerated aging phenotypes reflect a relevant biological role for the APC in aging, we assayed replicative and chronological aging in three additional mutants (apc9Δ, apc10Δ, and cdc26Δ), and in two different genetic backgrounds. The replicative life-span results (Figure 1, C and D) demonstrate...
that the three additional APC mutants also produced fewer daughters compared to wild-type cells. In Figure 1C, the data produced with the ResGen background are presented. The sgs1Δ mutant was included in this analysis, as Sgs1p, an ATP-dependent DNA helicase, is the yeast homolog of the human premature aging Werner syndrome protein and confers a severe replicative aging defect (Sinclair et al. 1997; Lebel 2001; McVey et al. 2001). The apec10Δ mutant was as defective as the sgs1Δ mutant. The results were identical when conducted in our laboratory S288c background (Figure 1D). The order of severity, from least to most impaired, was cdc26Δ, apec9Δ, and apec10Δ in both backgrounds, with the apec5Δ mutation conferring a life span similar to the apec9Δ mutation. Interestingly, the apec10Δ mutant was the only strain to generate the same replicative life-span curve in both the ResGen and S288c backgrounds. Note the extended life span observed in the ResGen background. The maximum life span observed using the ResGen BY4741 wild-type strain was 76 daughters (Figure 1C) and in the isogenic snf4Δ mutant the maximum achieved was 89 (data not shown). This is significantly greater than that observed for our laboratory S288c strain and other published strains (Kennedy et al. 1994; Ashrafi et al. 2000; Lin et al. 2002; Howitz et al. 2003). The BY4741 wild-type strain also achieved greater optical densities than any other wild-type strain we tested when grown at 30°C and 37°C (T. A. A. Harkness, unpublished results). Nonetheless, consistent with the replicative aging results, the apec mutants also conferred reduced chronological life spans (Figure 1E). The cdc26Δ mutant was again the least affected. Our results therefore demonstrate that the APC is required for prolonged aging in yeast.

As Cdc26p, Apc9p, and Apc10p are nonessential APC components and apec5Δ retains some function, it is possible that the different APC mutants cripple, but do not completely impair, the activity of a single aging pathway. Equally feasible is the possibility that different APC subunits contribute differently to the aging pathway. To distinguish between these ideas, we assayed apec5Δ apec9Δ, apec5Δ apec10Δ, and apec5Δ cdc26Δ double mutants for replicative life span. Only the apec5Δ apec10 double-mutant life span is reduced beyond that observed for either single mutant (Figure 2). These results suggest that Apc3p/Apc9p/Cdc26p and Apc10p define two separate APC subcomplexes that together act to extend life span in yeast.

Overexpression of APC10 is sufficient to increase life span: Next, we asked if the APC promotes life-span ex-
tension, would overexpression of APC subunits slow aging? Thus, wild-type cells were transformed with plasmids expressing APC5 or APC10 under the control of the CUP1 promoter. The transformants were then tested for replicative life span in the presence and absence of copper on SD-leu plates. Cells expressing the empty vector exhibited the same life span regardless of whether copper was present in the media (Figure 3). Cells expressing APC5, however, experienced a reduced life span, which was further reduced upon APC5 induction. Thus, Apc5p is a critical stoichiometric component of the APC complex, as both elevated and reduced levels of Apc5p shortened life span. Cells expressing APC10, on the other hand, had a markedly opposite effect. Expression of APC10 had no effect on life span in the absence of copper, but when induced, the maximum life span was increased over 50%. This effect was observed in both YTH3 and YTH6 wild-type cells. Thus, the APC is required for extended life span and the overexpression of just one of the 13 APC subunits in yeast is sufficient to slow aging.

**Figure 3.** Overexpression of APC10 increases the replicative life span of wild-type cells. Wild-type cells (YTH6) were transformed with the empty vector pYEX, which encodes the copper-inducible CUP1 promoter, or with pYEX containing APC5 or APC10. The transformants were then struck out onto SD-leu plates in the presence and absence of 0.5 mM CuSO4, and starting mothers were picked. The number of mothers assayed were as follows: WT + pYEX-Cu, 31; WT + APC5-Cu, 31; WT + APC10-Cu, 32; WT + pYEX + Cu, 32; WT + APC5 + Cu, 32; and WT + APC10 + Cu, 32.

**Multicopy suppression of apc5Δ by PDE2 and MIG1:** To gain insight into how APC-dependent aging is regulated, we performed a multicopy suppressor screen of the apc5Δ ts phenotype. PDE2 and MIG1 were recovered in this screen. The recovery of PDE2, a phosphodiesterase that degrades cAMP to downregulate PKA signaling, was not a surprise as PDE genes had already been shown to suppress ape mutations in multiple organisms (Yamasita et al. 1996; Heo et al. 1999; Irniger et al. 2000; Bolte et al. 2003). The recovery of PDE2 therefore validated our screen and provided evidence that biologically relevant factors were isolated. However, Mig1p, a glucose-responsive transcriptional modulator inhibited by the Snf1p kinase (Treitel et al. 1998; DeVit and Johnston 1999), had not previously been linked with the APC. Interestingly, the PKA and Snf1p kinase pathways had been shown to act antagonistically, as PDE2 suppressed snf1 phenotypes when expressed in multicopy (Hubbard et al. 1992).

To confirm that Mig1p activity was involved in APC function, a 2μ plasmid expressing MIG1 was obtained (a generous gift from H. Ronne). YEpl-MIG1 suppressed both the 37°C growth and chromatin-assembly defects associated with the apc5Δ strain (Figure 4). Single-copy expression of MIG1 also suppressed the apc5Δ ts phenotype (data not shown; the CEN-MIG1-GFP plasmid was kindly provided by M. Johnston). Since Mig1p is known primarily as a glucose-responsive transcriptional repressor, our results suggest that increased repression of a gene(s) by Mig1p overcomes the defects imposed by the apc5Δ allele. It has been shown that glucose downregulates the APC and that this effect is mediated by RAS/PKA signaling (Kotani et al. 1998; Irniger et al. 2000; Bolte et al. 2003). Therefore, Mig1p is in a critical position, as a glucose-responsive transcriptional repressor, to control glucose-regulated APC activity.

To determine whether the suppression of apc5Δ by increased expression of MIG1 reflects a relevant biological function for Mig1p in APC activity, Mig1p was disrupted in wild-type and apc5Δ cells by crossing the appropriate strains (the mig1Δ::LEU2 strain was generously supplied by M. Carlson). Strains expressing the mig1Δ::LEU2 allele...
Figure 4.—Multicopy expression of MIG1 suppresses the ts and chromatin-assembly-defective phenotypes associated with apc5Δ cells, but the mig1Δ mutation does not genetically interact with apc5Δ. (A) MIG1 multicopy expression restores growth to apc5Δ cells at 37°. apc5Δ cells (YTH1155) expressing multicopy MIG1 or the empty vector were grown at 30° and 37° on SD-leu plates for 3 days. (B) apc5Δ cells (YTH455) expressing MIG1 are chromatin assembly competent. Whole-cell extracts were prepared from apc5Δ cells transformed with 2μ-MIG1 or the empty vector control. In vitro supercoiling assays were performed with these extracts. Extracts derived from wild-type cells served as a control. (C) The mig1Δ mutation does not genetically interact with apc5Δ. Wild-type (YTH1480), apc5Δ (YTH1155), mig1Δ (YTH1482), and apc5Δ mig1Δ (YTH1484) cells were grown overnight in rich media. A 10-fold dilution series was prepared starting with 2 x 10^7 cells/ml and 5 μl of cells were spotted onto glucose-, galactose-, or glycerol-containing media. The plates were grown from 3 to 5 days at 30° and 37°. (D) mig1Δ cells exhibit in vitro chromatin assembly defects. Whole-cell extracts were prepared from the cells described in C and used to assay in vitro chromatin assembly as described in B.

Snf1p, the Mig1p inhibitor, functions together with the APC: To characterize the MIG1/apc5Δ interaction further, we investigated whether components that regulate Mig1p activity and localization genetically interact with apc5Δ. If increased expression of MIG1 suppressed apc5Δ phenotypes in a biologically relevant manner, then mutations to factors that inhibit Mig1p function, such as Snf1p, Snf4p (activator subunit of the Snf1 complex), and Msn5p (a nuclear membrane transporter required for nuclear export of Mig1p; DeVit and Johnston 1999), would also be expected to suppress apc5Δ defects. We first focused on whether snf1Δ and apc5Δ genetically interact. If Snf1p were the sole inhibitor of Mig1p activity, one consequence of disrupting SNF1 would be elevated Mig1p activity. Thus, disruption of SNF1 would be expected to suppress apc5Δ phenotypes. Surprisingly, our results showed that disruption of SNF1 exacerbated the apc5Δ ts defect on glucose and galactose (Figure 5A). One interpretation of this result is that Snf1p and the APC function together. Furthermore, since snf1Δ exacerbates the apc5Δ ts phenotype on glucose, Snf1p must be active on glucose. If Snf1p and the APC do function together, then snf4Δ and msn5Δ mutations would also be expected to exacerbate the apc5Δ phenotype. As predicted, snf4Δ and msn5Δ mutations worsen the apc5Δ ts phenotype on glucose (Figure 5B). Next, we investigated whether disruption of the
Figure 5.—The Snf1 complex and pathway components are required to activate the APC. (A) Snf1p positively influences APC activity. Fresh cultures of wild-type (YTH1472), snf1Δ (YTH1471), apc5Δ (YTH1470), and apc5Δ snf1Δ (YTH1469) cells were spotted onto glucose- or galactose-containing media in 10-fold dilutions so that the most concentrated spot contained 10⁵ cells. The plates were then incubated for 3 days at 30°C or 8 days at 37°C. After 8 days apc5CA cells will grow at 37°C. (B) Wild-type (YTH6), apc5CA (YTH1155), sip2Δ (YTH1402), apc5Δ sip2Δ (YTH1401), msn5Δ (YTH1403), apc5Δ msn5Δ (YTH1389), snf4Δ (YTH1611), and apc5Δ snf4Δ (YTH1520) cells were spot diluted, as in A, onto media containing glucose or galactose as a carbon source. The plates were then grown at 30°C or 37°C. The glucose plates were grown at 37°C for an extended period to observe the additive apc5CA msn5Δ and apc5Δ snf1Δ growth defects. (C) The snf1Δ strain (YTH1363) was transformed with plasmids overexpressing APC5 and APC10 from the CUP1 promoter (isolated from the ResGen GST-tagged library) with single-copy SNF1 and SNF4 (generous gifts from M. Carlson) and the empty vector YCp50. The transformant were grown on glucose- and galactose-containing media to test for suppression of the snf1Δ gal− phenotype.

Sip2p β-subunit of the Snf1 complex, which is believed to repress Snf1p (Ashraf et al. 2000; Lin et al. 2003), had an opposite effect on apc5-ca cells growth. Consistent with our hypothesis that Snf1p and the APC function together, the sip2Δ mutant completely suppressed the apc5-ca phenotype, but only on galactose (Figure 5B). The observation that sip2Δ is epistatic to apc5-ca strongly suggests that Sip2p and at least Apc5p have antagonistic effects, adding further strength to the hypothesis that the snf1Δ/ apc5-ca additive phenotype reflects a meaningful genetic interaction. Lastly, these data indicate that Mig1p has a negative effect on apc5-ca cells.

If Snf1p function is required for APC activity, then overexpression of APC subunits in snf1Δ cells may alleviate snf1Δ phenotypes, such as the inability to grow on galactose-containing media. This prediction was borne out as overexpression of APC5 and APC10 restored the snf1Δ gal− phenotype (Figure 5C). APC5 and APC10 expression is under the control of the CUP1 promoter in these plasmids, but copper was not required for adequate expression for suppression of the snf1Δ gal− phenotype. Furthermore, suppression of snf1Δ by both APC5 and APC10 suggests that the APC complex, and not just Apc5p, is a downstream effector of the Snf1p kinase. In summary, the above experiments strongly suggest that Snf1p is required for APC activity.

Expression of APC4 and APC9 is glucose regulated: Our data suggested that Snf1p and Mig1p are required for APC function. We were therefore compelled to test whether APC genes are regulated at the transcriptional level by glucose, as Snf1p, Mig1p, and APC activity are all influenced by glucose. We predicted that if APC genes were regulated by glucose at the transcriptional level, then Mig1p would be involved. Thus, we searched for potential Mig1p-binding sites in the promoters of APC and APC-activating genes (Fujibuchi et al. 2001). The search revealed 334 promoters that encode potential Mig1p sites from 6386 genes (5.2%). A previous
search of promoter sites turned up only 100 promoters (Lutfiyya et al. 1998). Four of the seven promoters tested from the Lutfiyya et al. (1998) study were glucose repressed and Mig1p and Mig2p dependent. The same study performed a microarray analysis using mRNAs isolated from a mig1Δ mig2Δ mig3Δ mutant. From ~325 genes included in the microarray, 235 genes were upregulated, while 38 were downregulated. APC genes were not identified in that study (M. Johnston, personal communication). One downregulated gene was confirmed to be glucose activated and this was Mig1p and Mig2p dependent. Thus, the Mig1p-like transcriptional repressors are required for the activation of some genes. Additional DNA microarray data obtained from mig1Δ mig1 Candida albicans cells demonstrated that of 2002 genes sampled (~25% of the genome), ~10% were derepressed (Murad et al. 2001). Therefore, the number of Saccharomyces cerevisiae Mig1p-binding sites reported is not likely an overestimate.

We found three APC genes to contain potential Mig1p-binding sites: APC1, APC4, and APC9. The Mig1p-repressed gene SUC2 contains two Mig1p sites within its promoter (Figure 6A). The consensus Mig1p site comprises an AT-rich stretch adjacent to a CG-rich segment. (B) WT (YTH1636), mig1Δ (YTH1482), mig2Δ (YTH1483), and mig3Δ (YTH1486) cells were grown in either 4% glucose (repressed) or 5% glycerol and 0.1% glucose (derepressed) and prepared for Northern analyses. A total of 30 μg of RNA was separated in each lane. Probes against APC4 and APC9 were prepared. Probes against SUC2 and RDN1 were used as positive and negative controls, respectively. In the experiment shown, the RDN1 blots were exposed for 1 hr and the SUC2, APC4, and APC9 blots were exposed for 48 hr. (C) WT (YTH1155) and apc5Δ (YTH1155) cells were transformed with YEp-MIG1 (pJO114) or the empty vector YEpplac111. The cells were grown under repressing conditions (SD-leu, 4% glucose) and as controls we used W2, mig1Δ, mig2Δ, mig3Δ, and mig1Δ mig2Δ (YTH1235) and apc5Δ (YTH1155) cells were transformed with YEp-MIG1 (pJO114) or the empty vector YEpplac111. The cells were grown under repressing conditions (SD-leu, 4% glucose) and prepared for a Northern analysis using the probes described above. The RDN1 blot was exposed for 2 hr while the SUC2, APC4, and APC9 blots were exposed for 48 hr. In B and C, native blots were used to probe for APC4 and APC9. These blots were then stripped and probed with either RDN1 or SUC2.

**Figure 6.**—Transcriptional control of APC genes. (A) Promoters of select APC genes contain putative Mig1p-binding sites. A search of all promoters within the yeast genome identified Mig1p sites within the APC1, APC4, and APC9 promoters. The putative sites are aligned with the two known Mig1p sites within the promoter of SUC2. The Mig1p site is defined by an AT-rich region adjacent to a CG-rich segment. (B) WT (YTH1636), mig1Δ (YTH1482), mig2Δ (YTH1483), and mig1Δ mig2Δ (YTH1486) cells were grown in either 4% glucose (repressed) or 5% glycerol and 0.1% glucose (derepressed) and prepared for Northern analyses. A total of 30 μg of RNA was separated in each lane. Probes against APC4 and APC9 were prepared. Probes against SUC2 and RDN1 were used as positive and negative controls, respectively. In the experiment shown, the RDN1 blots were exposed for 1 hr and the SUC2, APC4, and APC9 blots were exposed for 48 hr. (C) WT (YTH1155) and apc5Δ (YTH1155) cells were transformed with YEp-MIG1 (pJO114) or the empty vector YEpplac111. The cells were grown under repressing conditions (SD-leu, 4% glucose) and prepared for a Northern analysis using the probes described above. The RDN1 blot was exposed for 2 hr while the SUC2, APC4, and APC9 blots were exposed for 48 hr. In B and C, native blots were used to probe for APC4 and APC9. These blots were then stripped and probed with either RDN1 or SUC2.

**Mig1p and Mig2p are required for normal expression of APC4 and APC9:** The observation that at least APC4 and APC9 transcription is repressed by glucose is consistent with our hypothesis that Mig1p controls APC gene expression. To characterize the role that Mig1p plays in APC gene expression, APC4 and APC9 expression was analyzed in mig1Δ mutant cells. Since Mig1p transcriptional modulation is known to function redundantly with Mig2p (Lutfiyya et al. 1998), we also analyzed transcripts prepared from mig2Δ and mig1Δ mig2Δ cells. We observed that Mig1p is not involved with glucose repression of APC4 and APC9, as there was no difference in APC4 and APC9 transcript levels when isolated from wild-type and mig1Δ cells (Figure 6B; compare lanes 1 and 2). However, Mig2p is required for normal APC9 expression on glucose. However, the function of Mig2p is not as a repressor, but as an activator (Figure 6B; compare lanes 1 and 3). Furthermore, APC9 identifies a target that is acted upon by Mig2p indepen-
and 8). Mig1p and Mig2p are also required for SUC2 expression. APC4 plays a redundant role in this interaction of Mig1p and Mig2p may have influenced this result. It is consistent with the idea that multiple APC sub-complexes exist, as different subunits are regulated differently.

Interestingly, both Mig1p and Mig2p were found to play a redundant role in APC4 and APC9 activation in glycerol (Figure 6B; compare lane 5 with lanes 6, 7, and 8). Mig1p and Mig2p are also required for SUC2 activation in glycerol, with Mig2p playing a larger role. Therefore, both Mig1p and Mig2p are required for APC function by controlling the transcription of at least APC4 and APC9 in the absence of glucose.

Increased expression of MIG1 increases APC4 and APC9 expression: To confirm that Mig1p acts as an activator of APC4 and APC9 transcription, we expressed MIG1 in multicopy in wild-type and apc5CA cells, and determined the expression of APC4 and APC9 by a Northern analysis (Figure 6C). In both wild-type and apc5CA cells, increased expression of MIG1 resulted in increased expression of APC4 and APC9. Expression of SUC2 also increased when MIG1 expression was increased, as previously noted (Trethewey and Carlson 1995). Thus, the results presented in Figure 6, B and C, are consistent with the idea that Mig1p and Mig2p are required for the activation of at least APC4 and APC9.

Multicopy expression of MIG1 reduces the replicative life span: Both a decrease and an increase in Snf1p and APC activity reduces life span (Figures 1A and 3; data not shown; Ashrafi et al. 2000; Lin et al. 2003). We have also observed decreased chronological life span in snf1Δ and snf4Δ cells (data not shown). This likely reflects the fact that APC and Snf1p expression is tightly controlled; APC transcripts are exceedingly rare (data not shown; Figure 6B) and Snf1p protein levels are dramatically reduced on glucose (Vincent et al. 2001). The observation that increased APC10 expression prolongs life span (Figure 3) tipped the scales in favor of a role for the APC and Snf1p in life-span extension. We therefore turned to MIG1 and MIG2 to determine how Snf1p and the APC influence life span. Although a previous study found that mig1Δ had no effect on replicative aging (Ashrafi et al. 2000), we suspect that if Snf1p and the APC are required for life span, then Mig1p will have a negative effect on life span for two reasons: (i) Snf1p is a negative regulator of Mig1p, and (ii) the redundant interaction of Mig1p and Mig2p may have influenced the results reported by Ashrafi et al. (2000).

Mig1p functions to promote shortened chronological life span: To demonstrate that Mig1p reduces longevity through the APC, replicative and chronological life span was measured in apc5CA mig1Δ cells. As found for the ts and chromatin assembly apc5CA phenotypes (Figure 4, C and D), disruption of MIG1 had no effect on apc5CA replicative life span (data not shown). As reported previously (Ashrafi et al. 2000), the mig1Δ mutation also had little or no effect on an otherwise wild-type replicative life span and we show that mig1Δ had no effect on chronological life span (Figures 7B and 8C). However,
disruption of MIG1 completely restored the apcSΔA chronological aging defect (Figure 7B). The epistatic interaction observed between apcSΔA and migΔ, in regard to chronological aging, indicates that Mig1p may be the sole inhibitor of the APC-dependent chronological aging activity. Thus, the role Snf1p plays in at least chronological aging is consistent with Snf1p phosphorylation and inhibition of Mig1p, resulting in APC-dependent life-span extension.

**Mig2p activity accelerates aging:** Our results suggest that Mig1p functions redundantly with another factor to control replicative aging. An obvious candidate is Mig2p on the basis of our observation that Mig2p activates APC4 and APC9 transcription (Figure 6B). We first asked what affect disruption of MIG2 had on the apcSΔA ts phenotype. Figure 8A shows that disruption of MIG2 partially suppressed the apcSΔA ts defect, indicating that Mig2p has a negative effect on Apc5p function. However, in a mig2Δ background, disruption of MIG1 exacerbates the apcSΔA phenotype (Figure 8A). This is consistent with the notion that normal Mig1p function requires the presence of Mig2p. We next asked what effect disruption of MIG2 has on replicative life span. Strikingly, MIG2 disruption extends life span in both APC5 and apcSΔA cells to the same extent (Figure 8B). This indicates that Mig2p normally reduces life span and that reduced apcSΔA life span requires Mig2p. Finally, we asked what effect MIG1 disruption had on prolonged mig2Δ life span. The results in Figure 8C show that disruption of MIG1 in mig2Δ cells partially reduced the extended life span, while disruption of MIG1 in apcSΔA mig2Δ cells returned life span to wild type. Therefore, in the absence of MIG2, prolonged life span depended on Mig1p. This was particularly evident in apcSΔA mig2Δ cells. Taken together, our results describe a complex pathway of genetic interactions involving the Snf1p kinase, the downstream Snf1p target Mig1p, the Mig1p partner Mig2p, and a target of Mig1p/Mig2p activity, the APC, on prolonged longevity (Figure 9).

**DISCUSSION**

**Two aging pathways converge on the APC:** This report describes two antagonistic aging pathways that converge on the central cell-cycle regulator, the APC, to modulate life span (reviewed in Zachariae and Nasmith 1999; Harper et al. 2002). One pathway, consisting of the RAS/PKA-signaling molecules, has already been demonstrated to inhibit APC activity (Kotani et al. 1998; Irniger et al. 2000). The second pathway, in which the Snf1p kinase is a predominant player, was not known to interact with the APC, but has been shown to antagonize PKA activity (Hubbard et al. 1992; Hardy et al. 1994). On the basis of two initial sets of observations, we proposed that the Mig1p transcriptional repressor links the APC with Snf1p: (i) increased MIG1 expression suppressed apcSΔA phenotypes (Figure 1) and (ii) Snf1p represses Mig1p activity (Treitel et al. 1998). Considering that PKA activity inhibits the APC and reduces life span (Kotani et al. 1998; Jazwinski 1999; Lin et al. 2002), an antagonistic interaction between Snf1p and PKA (Hubbard et al. 1992) would suggest that Snf1p may have a positive influence on both APC activity and life span. Consistent with this hypothesis, we discovered that the APC is critical for prolonged life span (Figures 1–3). Thus, although both increased and decreased Snf1p expression reduces life span (Ashraf et al. 2000; Lin et al. 2003), the true role of Snf1p is likely to promote life-span extension.

**The Snf1p pathway extends life span:** The results presented in this study are consistent with the requirement of the Snf1p pathway for prolonged longevity. For
Figure 9.—A model depicting the possible molecular interactions controlling APC-dependent aging. Phosphorylation events are marked with a circled P. In the presence of glucose, Mig1p is imported into the nucleus and dephosphorylated. Under the same conditions, Snf1p is presumably dephosphorylated and transported out of the nucleus. The model predicts that when glucose is present, the APC is inhibited via a two-layered system; APC genes are glucose repressed and PKA inhibits the assembled APC complex. An unidentified factor (X), not Mig1p or Mig2p, is required for glucose repression of APC4 and APC9. Mig2p activates APC9 on glucose while both Mig1p and Mig2p activate APC4 and APC9 transcription on glycerol. Increased APC4 and APC9 expression is correlated with reduced life span, indicating that low-level APC activity is required for prolonged longevity. Increased activity of both PKA and Mig1p lead to a shortened life span. Interactions demonstrated in other labs are shown in black, those discovered in this report are shown in red, and interactions that we speculate upon are shown in green. Increased glucose conditions are shown in brown, whereas decreased glucose is shown in blue. Our results suggest that the level of complexity of this system is greater than that depicted.

APC activity is regulated by the Snf1p kinase pathway: Three sets of observations link Snf1p with APC activity: (i) both Snf1p and Snf4p are required for prolonged chronological life span (data not shown); (ii) Mig1p inhibits both chronological and replicative life-span extension (Figure 7); (iii) Mig2p reduces at least replicative life-span extension (Figure 8); and (iv) the APC is required for both chronological and replicative life-span extension (Figure 1). However, contrary to our hypothesis that Snf1p prolongs life span, two previous reports suggested that Snf1p activity shortened rather than extended life span (Ashrafi et al. 2000; Lin et al. 2003). For example, (i) Snf1p activity, as measured by histone H3 phosphorylation, increased in aging cells; (ii) increased expression of SNF1 resulted in phenotypes associated with accelerated aging; and (iii) increasing Snf1p activity by deleting SIP2 reduced replicative life span. It remains possible that these observations are a result of altering the presumed tight stoichiometric balance of Snf1p in the cell, as mentioned above, resulting in reduced life span. Furthermore, alternative interpretations can be envisioned for the observation that aging cells have increased Snf1p histone H3 kinase activity (Lin et al. 2003). On the one hand, phosphorylation of H3 and Mig1p by Snf1p may be regulated differentially. On the other hand, increased Snf1p activity in aging cells could in fact have the effect of keeping cells alive. Snf1p could be countering the effects of aging by phosphorylating and inhibiting Mig1p.

The involvement of glucose signaling in APC-dependent life span: The APC and the Snf1p and PKA-signaling pathways are all regulated by glucose (Kotani et al. 1998; Carlson 1999; Thevelein and de Winde 1999; Irniger et al. 2000; Schuller 2003). There are clear examples of conserved genes that are involved in glucose signaling affecting life span in model systems ranging from yeast to mice. An example is the insulin/IGF-1 pathway in higher eukaryotes, which is analogous to the glucose-signaling pathway in yeast controlled by RAS/ PKA (reviewed in Longo and Finch 2003). Mutations
that downregulate insulin signaling, like those that downregulated RAS/PKA signaling, increase life span. Starvation signals, whether derived from caloric restriction or nutrient limitation, shut down the glucose- and insulin-signaling pathways, causing the observed life-span extension. The extended life span afforded by caloric restriction has been shown to depend on Sir2p (Lin et al. 2000), a key regulator of life-span extension in yeast. Increased expression of Sir2 in Caenorhabditis elegans and in human cells also extended life span (Guarente and Kenyon 2000; Tissenbaum and Guarente 2001; Langley et al. 2002). Thus, it is tempting to speculate that Sir2p is linked with glucose signaling and APC activity in life-span determination. It will be interesting to test whether caloric restriction is also dependent upon APC activity.

**Mig1p and Mig2p are required for APC4 and APC9 transcription:** Our observation that both Mig1p and its redundant partner Mig2p shorten life span supports our hypothesis that Snf1p promotes longevity. Our results demonstrate that not only is APC activity inhibited by glucose-induced phosphorylation of APC subunits by PKA (Kotani et al. 1998; Irniger et al. 2000), but also APC gene transcription is glucose repressed (Figure 6B). A search of the yeast genome revealed that the promoters of three APC genes, APC1, APC4, and APC9, contain Mig1p-binding sites (Figure 6A; Fujibuchi et al. 2001). The three APC genes harbored only one copy of the consensus site, compared to two sites in the glucose- and Mig1p-repressed SUC2 gene, and they were found at a distance from the transcript start site (350–650 bp) similar to that for the SUC2 sites (∼450 bp). A direct analysis of APC transcripts revealed them to be exceedingly rare (Figure 6B; data not shown). Nonetheless, APC4 and APC9 transcripts were repressed on glucose. Consistent with only one Mig1p site in APC genes, compared to two in SUC2, glucose repression was more dramatic for SUC2 expression. Interestingly, neither Mig1p nor Mig2p was required for glucose repression of APC4 and APC9. Mig2p, however, activated transcription of APC9 on glucose, while Mig1p had no effect. This is the first demonstration of a gene regulated by Mig2p independently of Mig1p (Lutﬁyya et al. 1998). This likely explains why Mig2p plays a larger role than Mig1p in reducing life span on glucose (Figure 8). Furthermore, neither Mig1p nor Mig2p affected APC4 transcription on glucose. This is the first indication that different APC genes are regulated by dramatically different mechanisms. These data suggest that additional factors capable of glucose repression of APC genes remain to be characterized. We are presently pursuing the identity of these factors.

Our results demonstrate that both Mig1p and Mig2p are required for the activation of APC4 and APC9 on glycerol (Figure 6B). In glycerol, it is presumed that the APC is at least not inhibited, as APC mutants are suppressed on glucose-free media, such as glycerol (Figure 4C) and raffinose (Irniger et al. 2000). On the basis of our present data, we conclude that activation of APC4 and APC9 by Mig1p and Mig2p may play a positive role in APC function in glycerol-grown cells. However, future investigation will be required to resolve this issue, as apc5Δ mig1Δ mig2Δ cells grow similarly to wild type on glycerol at 37°C (data not shown), suggesting that Mig1p and Mig2p may continue to be a negative influence on APC activity. Contrary to the literature, it is interesting to note that Mig1p retains activity on glycerol. We also observed that the snf1Δ mutation has its greatest effect on the apc5Δ ts phenotype on glucose (Figure 5). Hence, these proteins are not strictly regulated by glucose. This has been observed previously as Snf1p activity increases in aging glucose-grown cells (Lin et al. 2003) and Mig1p and Mig2p continue to repress glucose-repressible genes even in glycerol (Lutfiyya et al. 1998). While Mig1 is known to be glucose repressed and inhibited by Snf1p (Lutfiyya et al. 1998; Treitel et al. 1998), future work will be required to elucidate the mechanisms controlling Mig2p activity, as it is neither glucose repressed nor Snf1p inhibited (Lutfiyya et al. 1998).

**Why does MIG1 expression suppress apc5Δ defects, yet reduce life span?** The observation that multicopy expression of MIG1 has opposite effects on the chromatin assembly and aging defects associated with apc5Δ presents us with an interesting challenge. One possible mechanism to explain these results is that Mig1p interacts with different target gene promoters in regard to its function in chromatin assembly and aging. Thus, perhaps Mig1p activates chromatin assembly, but accelerates aging. A second mechanism could involve the activation of genes normally repressed by Mig1p when MIG1 expression increases, as earlier proposed by Treitel and Carlson (1995). Although we observe that SUC2 expression is indeed increased as MIG1 expression increases, we do not observe this to be the case with APC4 and APC9 transcripts (Figure 6C). A third scenario could involve glucose. For example, glucose signaling stimulates growth, yet it shortens life span (Longo and Finch 2003). As Mig1p activity is glucose dependent, increased expression of MIG1 could mimic increased glucose signaling, thereby stimulating growth and shortening life span. Since Mig1p appears to be required for chromatin assembly, perhaps reflecting its role in growth stimulation, it is not surprising that increased MIG1 expression suppresses the apc5Δ chromatin assembly defect.

**A possible link connecting chromatin assembly with aging:** Chromatin metabolism is being described to play an ever-increasing role in life-span determination (Campisi 2000; Chang and Min 2002; Lin et al. 2003). We obtained additional evidence supporting the notion that aging and chromatin metabolism are linked. The observation that apc10Δ cells, which are also chromatin assembly defective (discussed in Harkness et al. 2002), senesced at the same rate as an sgs1Δ mutant (Figure 1C), is suggestive of the idea that Sgs1p and at least
Apc10p function together to determine aging. The Sgs1p protein is the yeast homolog of the human gene responsible for the Werner syndrome (Watt et al. 1996). Humans afflicted with Werner syndrome exhibit all the symptoms of advanced and accelerated aging (Lebel 2001; Ostler et al. 2002). The SGS1 gene encodes a member of the conserved RecQ family of DNA helicases that is required for at least the maintenance of the rDNA locus in yeast (Cobb et al. 2002; Kaliraman and Brill 2002). Hence, the early senescence observed in sgs1Δ mutants is believed to be in part due to the accumulation of ERCs (Sinclair et al. 1997; McVey et al. 2001).

A direct link between aging and chromatin, however, may come from the observation that the sgs1Δ mutant was found to be synthetically lethal when combined with a mutation to the chromatin assembly factor Asf1p (Tyler et al. 1999; Tong et al. 2001). We have recently observed that ASFI overexpression in apc5Δ cells suppressed the apc5Δ growth defect (T. A. A. Harkness, T. G. Arnason, C. Legrande and M. G. Piscilevich, unpublished results). It is tempting to speculate from these observations that the APC and Sgs1p may participate in a common aging pathway that recruits chromatin-modifying enzymes to specific chromosomal loci, such as the rDNA locus. However, direct evidence that conclusively links the APC chromatin assembly and aging pathways awaits the identification and characterization of additional APC targets.

**How would the APC be beneficial in times of need?**
Recent studies have demonstrated that caloric restriction enhances life span in almost all organisms studied (reviewed in Hekimi and Guarente 2003; Koubova and Guarente 2003; Longo and Finch 2003). Caloric restriction has been proposed to enhance life span as a means to allow organisms experiencing shortages in their environment to survive long enough to benefit when conditions again become bountiful. To achieve a similar end, diploid yeast, when nutrients are limiting, are induced to undergo sporulation (Destrueelle et al. 1994). Yeast spores, because of their tough cell walls, are highly resistant to many stresses and can remain dormant for long periods of time (Smits et al. 2001). Therefore, the induction of the APC in yeast when glucose is limiting, mediated perhaps by Mig1p and Mig2p, would ensure that all cells progress through exit mitosis to reach a stage in their life cycle where sporulation can be accomplished. Consistent with this model, we observe that heterozygous and homozygous apc5Δ diploid cells require more time to complete sporulation when compared to isogenic wild-type cells (T. A. A. Harkness, unpublished data).

**Future directions:** The striking observation that virtually all the proteins and functions studied in this report are evolutionarily conserved suggests a common aging pathway. Also, the recent reports demonstrating the importance of strict control of APC component expression and the onset of many diseases when this control goes awry are particularly relevant to this study. For example, the APC has been described as an important anticancer target (reviewed in Pray et al. 2002). Evidence supporting this claim comes from studies showing that the human T-lymphotropic virus type 1 Tax oncoprotein inappropriately activates the APC, likely playing a large role in the onset of viral adult T-cell leukemia (Liu et al. 2003). Alternatively, in some cancers APC activity is downregulated (Wang et al. 2003; Song et al. 2004). We expect to show in our future work that the APC-dependent aging pathway that we have characterized in yeast will also apply to higher eukaryotes. Understanding the molecular events in a simple model system will allow a rapid dissection of the mechanisms controlling life-span determination in higher vertebrates.

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