Genome instability is an important component of chronological aging in budding yeast [29] and other organisms [8]. To determine whether the less efficient growth arrest in G1 and shortened lifespan of nutrient-depleted cells ectopically expressing *CLN3* are accompanied by accelerated age-dependent genome instability, we asked whether these cells suffer an increase in the frequency of mutations in the *CAN1* gene, as measured by increased resistance to the toxic amino-acid analogue canavanine. We found that, similar to a previous report [29], nutrient depletion induced a chronological-age-dependent increase in mutation frequency in wild-type cells (Fig. 5D; "vector"). This mutation frequency was dramatically elevated in chronologically aged cells ectopically expressing *CLN3* (Fig. 5D; "pCLN3"). Therefore, in addition to shortening chronological lifespan and stimulating apoptosis, the failure to efficiently arrest growth in G1 during nutrient depletion contributes to chronological age-dependent genome instability.

DISCUSSION

Impact of altered nutrient signaling on chronological lifespan

Most efforts to understand how alterations in nutrient signaling pathways impact the chronological lifespan of budding yeast have focused on changes in oxidative stress responses regulated by these pathways downstream of Rim15. Our findings point to an efficient Rim15-dependent growth arrest in G1 that also requires down-regulation of Cln3 as an additional factor determining chronological lifespan in this organism (Fig. 6A). Caloric restriction, mutational inactivation of Sch9 or Ras2 and growth in YPD rather than SC medium enhance this G1 arrest and extend lifespan. In contrast, constitutive activation of nutrient signaling by $RAS2^{val19}$ or deletion of *RIM15* increases the frequency with which nutrient-depleted cells growth-arrest in S phase instead of G1 and shortens chronological lifespan. During nutrient depletion,

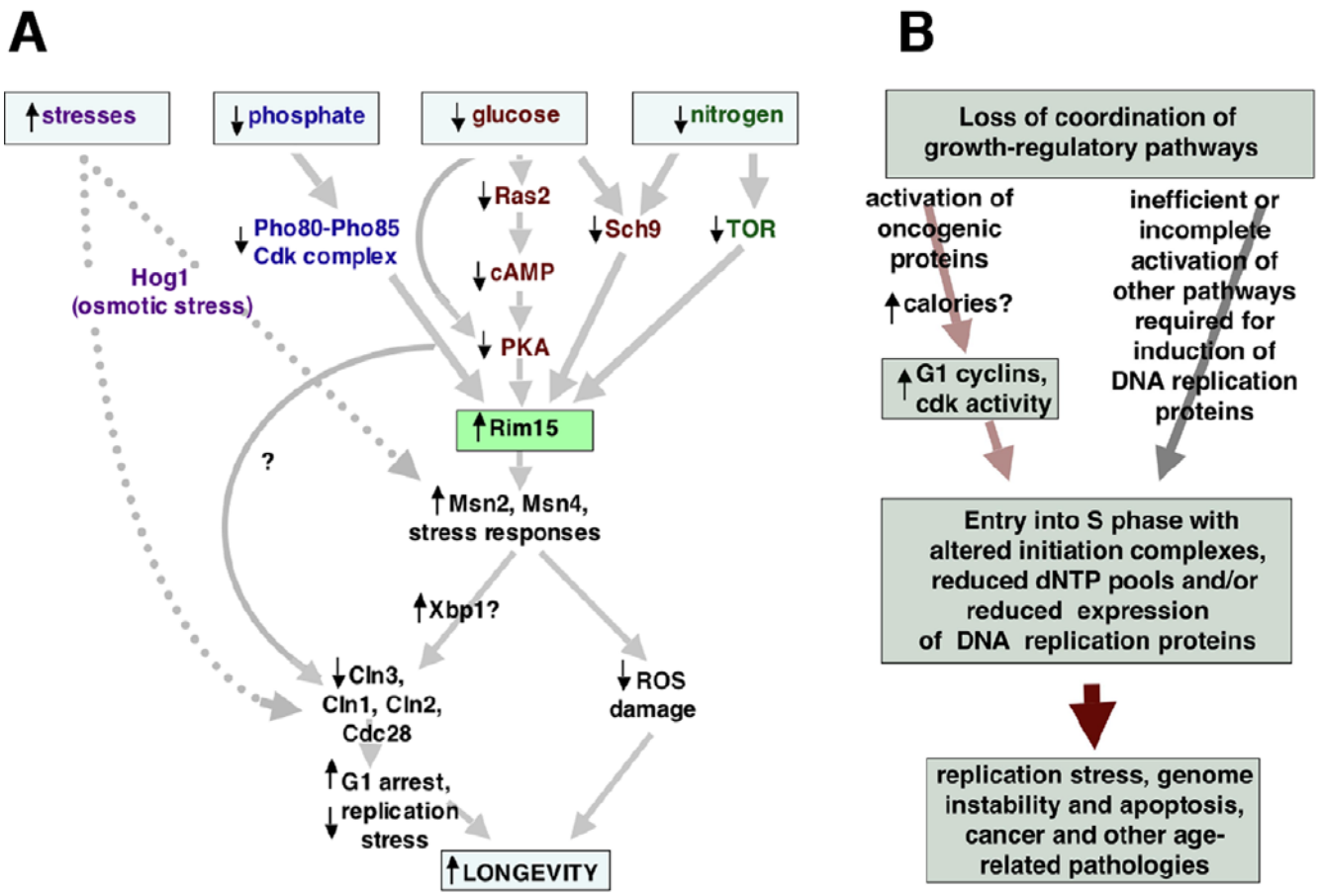

Figure 6. Models for longevity regulation by growth signaling pathways in budding yeast (A) and deregulation of growth regulatory pathways leading to replication stress and aging in all eukaryotes (B). A. In budding yeast, nutrient signaling pathways that respond to glucose, nitrogen and phosphate converge upon Rim15, which is downregulated by signaling through these pathways when nutrients are plentiful. Activation of Rim15 when nutrient signaling is inhibited induces stress response factors (including Msn2 and Msn4) and stress responses mediated by Sod1, Sod2 and other proteins that mitigate oxidative damage and other effects of stresses. Reduced nutrient signaling also downregulates Cln3 and downstream Clns1 and 2, which are required for activation of the cyclin-dependent kinase Cdc28. Inactivation of Cdc28 during nutrient depletion contributes to a G1 arrest that protects against replication stress. Osmotic stress (and perhaps other stresses) also contribute to G1 arrest during nutrient depletion. G1 arrest in response to nutrient depletion and other stresses may be mediated in part by induction of the transcriptional repressor Xbp1, which inhibits Cln transcription. Enhancement of G1 arrest by caloric restriction, osmotic stress or mutational inactivation of nutrient signaling pathways protects against replication stress and promotes longevity in combination with Rim15-dependent responses to oxidative-and other stresses. **B.** Replication stress associated with uncoordinated entry into or exit from S phase downstream of the activation of some, but not all, growth signaling pathways may be an important factor determining lifespan in all eukaryotes. In both panels, question marks indicate hypothetical or undefined effects.

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