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# Initiation of the yeast $G_0$ program requires Igo1 and Igo2, which antagonize activation of decapping of specific nutrient-regulated mRNAs

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**Growth factors and essential nutrients are key controllers of eukaryotic cell proliferation. In their absence, cells may enter into a quiescent ($G_0$) state. In yeast, nitrogen and/or carbon limitation causes downregulation of the conserved TORC1 and PKA signaling pathways and consequently activation of Rim15, a member of the PAS protein kinase family. Rim15 orchestrates the initiation of the $G_0$ program in part by coordinating transcription of Msn2/4- and/or Gis1-dependent genes with posttranscriptional protection of the corresponding mRNAs via direct phosphorylation of Igo1/2. Here, we show that several factors including Ccr4, the Lsm-Pat1 complex, and Dhh1, which are implicated in mRNA decapping activation, participate in the decay of specific mRNAs during initiation of the $G_0$ program when Igo1/2 are absent. Accordingly, Igo1/2 likely play a key role in preventing the decapping and subsequent 5'-3' degradation of a set of nutrient-regulated mRNAs that are critical for cell differentiation and chronological life span.**

**Key words:** quiescence, TORC1, mRNA stability, 5'-3' mRNA decay pathway, Igo1 and Igo2, yeast

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## Introduction

All living cells appear to be capable of exiting the normal cell cycle and entering a reversible state termed quiescence or G-zero ($G_0$), which is typically characterized by low metabolic activity, including low rates of protein synthesis and transcription. Initiation of the $G_0$ program is a highly coordinated process, which requires downregulation of conserved nutrient-, growth factor- and/or hormone-responsive signal transduction pathways. In the yeast *Saccharomyces cerevisiae*, downregulation of the nutrient-regulated kinase activities of the conserved *T*arget *O*f *R*apamycin *C*omplex 1 (TORC1), or the *p*rotein *k*inase *A* (PKA) had been found to drive cells into a $G_0$-like state and to significantly extend chronological life span (CLS).[1-4] In contrast, loss of the kinase activity of Rim15 prevents access to $G_0$ and decreases CLS.[5-7] TORC1 (via its substrate Sch9) and PKA are thought to signal in parallel pathways to positively regulate ribosome biogenesis and growth and, by maintaining Rim15 in an inactive state in the cytoplasm, to negatively regulate entry into $G_0$.[8-12] The molecular mechanisms linking Rim15 to distal readouts such as the expression of specific nutrient-regulated genes (e.g., *HSP26*; see below), trehalose and glycogen accumulation, and CLS extension involve the stress-response and post-diauxic shift transcription factors Msn2/4 and Gis1,[7,13,14] respectively, as well as the recently identified paralogous Rim15 target proteins Igo1 and Igo2.[15] Intriguingly, during initiation of the $G_0$ program, phosphorylation by Rim15 stimulates Igo proteins to associate with both Lsm12, which likely binds to the 3' *un*translated *r*egion (UTR) of mRNAs in a complex with Pbp1 and Pbp4,[16,17] as well as with the decapping activator Dhh1. This latter event appears to be key for newly expressed mRNAs to be both sheltered from degradation via the 5'-3' mRNA decay pathway and channeled towards stress granules (SGs) for

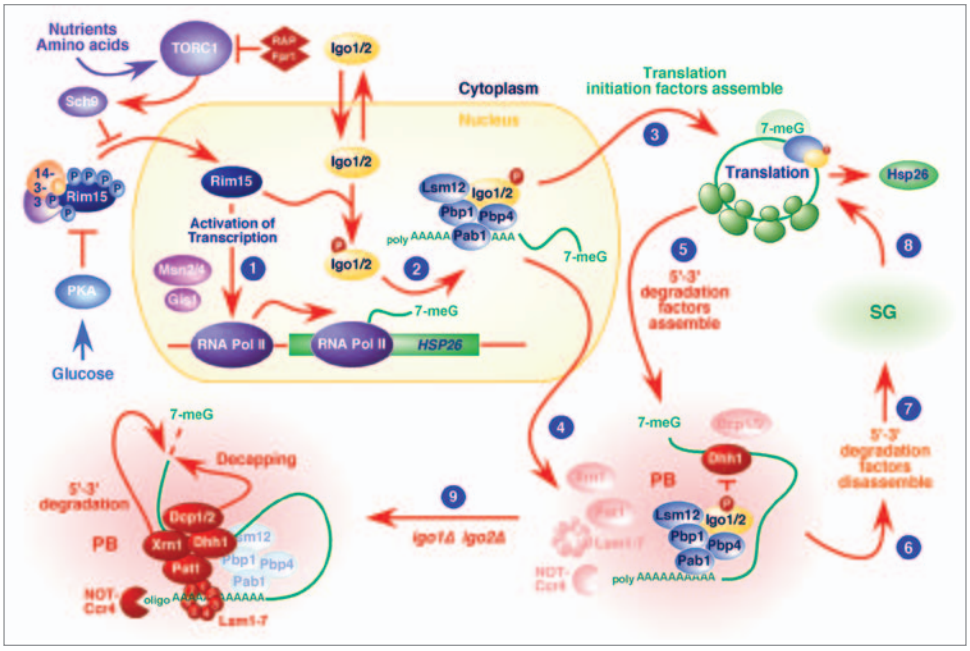


**Figure 1.** Model depicting a role for Igo1 and Igo2 in protection of specific mRNAs, which are induced during initiation of the $G_0$ program. In exponentially growing cells, phosphorylation of Rim15 (on $Ser^{1061}$ by the TORC1 target Sch9 and on $Thr^{1075}$ by the cyclin-cyclin-dependent kinase complex Pho80-Pho85)[8,10] mediates tandem 14-3-3 binding to guarantee optimal sequestration of Rim15 in the cytoplasm, where it is kept inactive through additional PKA-mediated phosphorylation events.[5,9] TORC1 inactivation (e.g., following treatment of cells with rapamycin or following nutrient starvation) causes initiation of the $G_0$ program in part by abrogating the cytoplasmic retention of Rim15. Nuclear Rim15, presumably released from PKA-mediated inhibition,[26] has a dual role in controlling gene expression by activating transcription of Msn2/4- and Gis1-dependent genes (1) and protecting the corresponding transcripts from degradation via an Igo1/2-dependent mechanism (2–7).[13-15] Accordingly, Rim15-dependent phosphorylation of $Ser^{64}$ in Igo1 and $Ser^{63}$ in Igo2 (both of which likely shuttle between the cytoplasm and the nucleus) leads to the formation of specific messenger ribonucleoprotein (mRNP) complexes (possibly already in the nucleus as depicted or, alternatively, in the cytoplasm) that contain at least Igo1/2, Lsm12, the poly(A)-binding protein Pab1 interactor Pbp1, and Pbp4, as well as a nutrient-regulated mRNA (e.g., *HSP26*) that needs to be translated as part of the quiescence program (2). To this end, the cytoplasmic mRNP complexes likely associate with translation initiation factors and ribosomes for direct translation (3). Alternatively, they may transiently assemble (to some extent in PBs) with 5'-3' degradation factors (4). Notably, Dhh1 may either be incorporated into *HSP26* mRNA-containing mRNPs while they reside in the nucleus, or—as depicted in our model—join the corresponding mRNPs in the cytoplasm (e.g., during or following their unloading from ribosomes and disassembly of translation initiation factors) (5). The interaction of p-$Ser^{64}$-Igo1 with Dhh1 presumably prevents decapping and subsequent 5'-3' degradation of the associated mRNA and promotes both disassembly of 5'-3' degradation factors from the mRNP (6) and reassembly of translation initiation factors (7), a process that may specifically occur in stress granules (SGs). Such mRNP complexes (that may still contain Igo1/2) are thought to exit SGs and to subsequently associate with ribosomes for translation of the corresponding mRNA (8). In the absence of functional Igo1/2, mRNAs such as the *HSP26* mRNA remain unguarded from Dcp1/2- and Xrn1-mediated decapping and 5'-3' decay (9), respectively, and are therefore prone to degradation.

subsequent translation (**Fig. 1**). Given the conserved nature of Igo proteins,[15] these findings indicate that stabilization of specific mRNAs is a critical determinant of cell differentiation and CLS.

## The mRNA Decapping Pathway Targets *HSP26* mRNAs in the Absence of Igo1 and Igo2

To further define the molecular function of Igo1/2, we performed a genome-wide screen for mutations that suppress the defect of *igo1*Δ *igo2*Δ cells in *HSP26* expression during initiation of the $G_0$ program. To this end, we constructed an *HSP26*-yEmRFP reporter gene that expresses, under the control of the yeast *HSP26* promoter, a new *y*east *e*nhanced variant of the *m*Cherry *m*onomeric *r*ed *f*luorescent *p*rotein (yEmRFP),[18] which confers a vivid purple color to wild-type cells during and following initiation of the $G_0$ program. As expected, only wild-type cells containing the *HSP26*-yEmRFP reporter plasmid (pXL029), but not corresponding *rim15*Δ or *igo1*Δ *igo2*Δ cells, were able to form purple colonies when grown for five days on plates containing synthetic defined (SD) medium (**Fig. 2A**). Next, we crossed an *igo1*Δ *igo2*Δ strain (XL111; MATα, *igo1*Δ*::NatMX4*, *igo2*Δ*::HphMX4*, *can1*Δ*::STE2pr-His5*, *lyp1*Δ, *ura3*Δ, *his3*Δ*1*; derived from strain Y7092; C. Boone lab collection), harboring the *HSP26*-yEmRFP reporter plasmid, with the Euroscarf collection of 4,857 viable gene deletion mutants essentially as described earlier.[19] The resulting triple mutant collection was screened for mutants that, unlike *igo1*Δ *igo2*Δ cells, were able to express *HSP26*-yEmRFP and consequently form purple colonies. Notably, since Rim15 controls both activation of transcription (via a still partially understood mechanism that implicates the transcription factors Msn2/4 and Gis1) and posttranscriptional stabilization of mRNAs (by a process that implicates Igo1/2) (**Fig. 1**), we reasoned that candidate mutations that

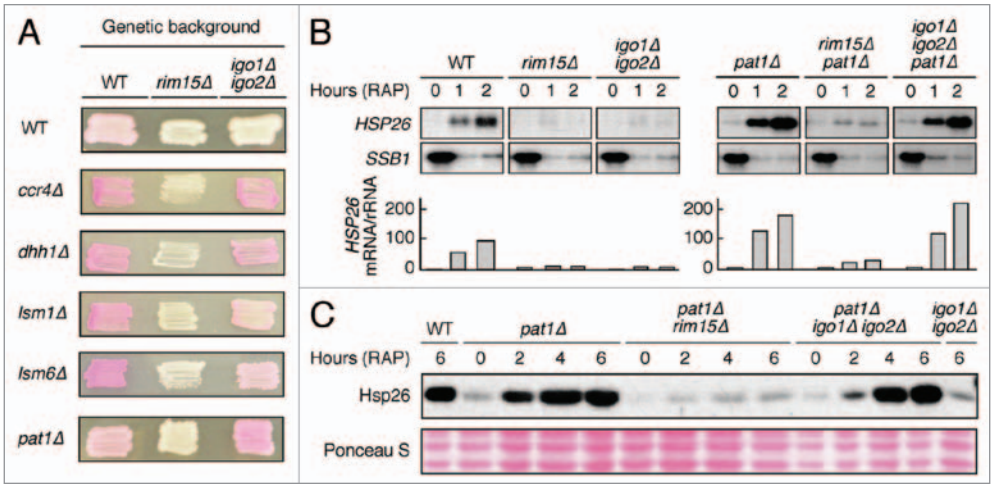


**Figure 2.** The 5'-3' mRNA degradation pathway targets *HSP26* mRNAs in the absence of Igo1/2. (A) Individual strains were constructed by backcrosses between the single knock-out strains from the Euroscarf collection and isogenic wild-type (BY4742), XL126 (MATα, *rim15*Δ::*NatMX6*) or XL122-16D (MATα, *igo1*Δ::*NatMX4*, *igo2*Δ::*HphMX4*) cells, transformed with the *HSP26*-yEmRFP reporter plasmid (pXL029), patched on SD-medium-containing plates, and grown for 5 days at 30°. Expression of yEmRFP under the control of the HSP26 promoter confers to cells a purple color. (B) Loss of Pat1 suppresses the defect of *igo1*Δ *igo2*Δ, but not that of *rim15*Δ cells in rapamycin-induced *HSP26* mRNA expression. Northern blot analyses of *HSP26* and of rapamycin-repressible *SSB1* were performed with wild-type and indicated mutant strains prior to (0) and following a rapamycin treatment (RAP; 0.2 mg $ml^{-1}$) of 1 hr or 2 hr. Bar graphs show the relative level of *HSP26* mRNA per rRNA (quantified by Phosphor-Imager analysis and arbitrarily set to 1.0 for exponentially growing wild-type cells). All samples were run on the same gel (identical film exposure time). (C) Loss of Pat1 fully suppresses the defect of *igo1*Δ *igo2*Δ, but not that of *rim15*Δ, in rapamycin-induced Hsp26 protein expression. Cell extracts from the same strains as in (B), treated (2 hr, 4 hr and 6 hr) or not (0 hr) with rapamycin, were analyzed by SDS-PAGE, and immunoblots were probed with specific anti-Hsp26 antibodies. Ponceau S staining of the membranes prior to immunoblot analysis serves as loading control.

suppress the *HSP26*-yEmRFP expression defect in both *rim15*Δ and *igo1*Δ *igo2*Δ cells are very likely to play a role in regulating transcription rather than mRNA stability. To more specifically focus on the role of Igo proteins in posttranscriptional mRNA stability control, such mutations were eliminated in a secondary screen following which we retained a total of five gene deletions that were able to suppress the defect of *igo1*Δ *igo2*Δ cells, but not that of *rim15*Δ cells. In line with and confirming our previous results,[15] our screen identified the *ccr4*Δ and *dhh1*Δ mutations, both of which prevent mRNA decay at early steps of the 5'-3' mRNA decay pathway,[20] as *igo1*Δ *igo2*Δ suppressor mutations (**Fig. 2A**). Interestingly, all three additional, newly identified suppressor mutations (i.e., *lsm1*Δ, *lsm6*Δ and *pat1*Δ) affect genes whose products are constituents of the Lsm1-7/Pat1 (Lsm-Pat1) complex that activates mRNA decapping following deadenylation.[21] Notably, the other subunits of this complex are either essential (Lsm2-5), or their absence did not significantly suppress (Lsm7) the *igo1*Δ *igo2*Δ phenotype in our assays (data not shown).

To assess further whether loss of Lsm-Pat1 function can also suppress the defect of *igo1*Δ *igo2*Δ cells in *HSP26* expression following TORC1 inactivation, we next measured *HSP26* mRNA and Hsp26 protein levels in rapamycin-treated wild-type, *rim15*Δ, *igo1*Δ *igo2*Δ, *pat1*Δ, *pat1*Δ *rim15*Δ and *pat1*Δ *igo1*Δ *igo2*Δ cells. As expected, loss of Pat1 fully suppressed the defect of *igo1*Δ *igo2*Δ cells, but not that of *rim15*Δ cells, in rapamycin-induced *HSP26* expression (both at the mRNA and protein levels; **Fig. 2B and C**). These results further support the idea that Rim15 likely has a dual role in controlling gene expression by (1) activating transcription (**Fig. 1**) and (2) protecting the corresponding transcripts from degradation via an Igo1/2-dependent mechanism that specifically antagonizes the process of mRNA decapping activation. In line with such a model, we previously found that loss of the 5'-3' exonuclease Xrn1, which causes processing bodies (PBs) to increase in number and size due to the entrapping of non-degraded mRNAs,[20] allowed *igo1*Δ *igo2*Δ cells, but not *rim15*Δ cells, to accumulate *HSP26* mRNAs, which then failed to be translated into proteins.[15]

mRNA decapping is thought to occur in multiple, sequential steps, which include the partial deadenylation of the poly(A) tail by the NOT-Ccr4 complex, recruitment to the corresponding oligoadenylated mRNAs of the Lsm-Pat1 complex—which, as recently shown in metazoans, serves as a scaffold that binds (via Pat1b) both deadenylation and decapping enzymes[22,23]—and Dhh1-mediated activation of decapping following remodeling of the 5' structure of the mRNA.[20,24] Since a defect in any of these steps in the mRNA decapping process can suppress the phenotype of *igo1*Δ *igo2*Δ cells in *HSP26* mRNA (and Hsp26 protein) expression and given the finding that Igo proteins (following phosphorylation by Rim15) interact with Dhh1,[15] Igo proteins are likely to inhibit mRNA decapping at a stage from which mRNAs can still successfully be retrieved for translation (following disassembly of 5'-3' decay factors and assembly of translation initiation factors that may possibly occur within stress granules) (**Fig. 1**).

Consistent with this view, Igo proteins appear to reach SGs following a transient stopover in PBs in glucose-limited cells.[15]

## Outlook

While our studies have pinpointed a role for Igo proteins in stabilization of nutrient-regulated mRNAs, which appears to be critical for both initiation of the $G_0$ program and proper setup of CLS,[15] they also rise a number of interesting issues to be tackled in the future. For instance, rapamycin-induced expression of the *HSP26-LacZ* and *HSP26-yEmRFP* reporter genes, like expression of *HSP26*, was strongly dependent on Igo1/2, indicating that either the promoter region or the 5' UTR of *HSP26* critically defines the fate of the corresponding mRNA by a process that implicates Igo1/2. One attractive model, to be tested in this context, is that Rim15 may be recruited to specific promoter regions to coordinate both temporal and spatial activation of Igo1/2 and ensure timely co-transcriptional loading of activated Igo1/2 onto Lsm12-, Pbp1- and Pbp4-containing messenger ribonucleoprotein (mRNP) complexes. An alternative model that can be experimentally addressed is that activated Igo1/2 may be able to (directly or indirectly) bind to a specific structure or sequence motif within the 5' UTR of nutrient-regulated mRNAs such as the *HSP26* mRNA. Moreover, our present findings suggest that Igo1/2 inhibit 5'-3' mRNA decay of mRNAs by interfering with the mRNA decapping activation system. Future studies that specifically focus on structural/functional aspects of the interaction between Igo1/2 and Dhh1 (and possibly the Lsm-Pat1 complex) are therefore very likely to significantly enhance our general understanding of the mechanisms that control mRNA decay. Finally, the closest homolog of Igo1/2 in mammals, i.e., ARPP-19, has previously been suggested to control axon growth and synaptic plasticity specifically by stabilizing the *g*rowth-*a*ssociated *p*rotein-43 (GAP-43) mRNA in response to nerve growth factor treatment.[25] Thus, an exciting challenge will be to test whether the homologs of Igo proteins in higher eukaryotes may have an evolutionarily conserved role in mRNA stability control, which—as observed in yeast—may be critical for cell differentiation and life span.


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