Computational and Structural Biotechnology Journal 20 (2022) 107–116

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Mini review

Yeast Crf1p: An activator in need is an activator indeed

Sanjay Kumar *, Muneera Mashkoor, Anne Grove *

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

ARTICLE INFO

Article history:
Received 28 August 2021
Received in revised form 15 November 2021
Accepted 3 December 2021
Available online 08 December 2021

Keywords:
Crf1p
Ifh1p
Gene regulation
mTORC1
Ribosomal protein
Ribosome biogenesis

ABSTRACT

Ribosome biogenesis is an energetically costly process, and tight regulation is required for stoichiometric balance between components. This requires coordination of RNA polymerases I, II, and III. Lack of nutrients or the presence of stress leads to downregulation of ribosome biogenesis, a process for which mechanistic target of rapamycin complex I (mTORC1) is key. mTORC1 activity is communicated by means of specific transcription factors, and in yeast, which is a primary model system in which transcriptional coordination has been delineated, transcription factors involved in regulation of ribosomal protein genes include Fhl1p and its cofactors, Ifh1p and Crf1p. Ifh1p is an activator, whereas Crf1p has been implicated in maintaining the repressed state upon mTORC1 inhibition. Computational analyses of evolutionary relationships have indicated that Ifh1p and Crf1p descend from a common ancestor. Here, we discuss recent evidence, which suggests that Crf1p also functions as an activator. We propose a model that consolidates available experimental evidence, which posits that Crf1p functions as an alternate activator to prevent the stronger activator Ifh1p from re-binding gene promoters upon mTORC1 inhibition. The correlation between retention of Crf1p in related yeast strains and duplication of ribosomal protein genes suggests that this backup activation may be important to ensure gene expression when Ifh1p is limiting. With ribosome biogenesis as a hallmark of cell growth, failure to control assembly of ribosomal components leads to several human pathologies. A comprehensive understanding of mechanisms underlying this process is therefore of the essence.

Contents

Abbreviations: CK2, casein kinase 2; Crf1, corepressor with forkhead like; FHA, forkhead-associated; FHB, forkhead-binding; Fhl1, forkhead like; Fpr1, FK506-sensitive proline rotamase; FKBP, FK506 binding protein; Hmo1, high mobility group; Ifh1, interacts with forkhead like; mTORC1, mechanistic target of rapamycin complex 1; Rap1, repressor/activator protein; RASTR, ribosome assembly stress response; RiBi, ribosome biogenesis; RP, ribosomal protein; Sfp1, split finger protein; WGD, whole genome duplication.

* Corresponding authors.
E-mail addresses: skuma28@lsu.edu (S. Kumar), agrove@lsu.edu (A. Grove).

https://doi.org/10.1016/j.csbj.2021.12.003

1. Introduction

Ribosomes are subcellular machines required for protein synthesis. The absolute requirement for ribosomes during the cellular lifespan makes their biosynthesis essential, and it is an energy-consuming process. For example, during normal growth of *Saccharomyces cerevisiae*, an average of ~ 2,000 ribosomes are assembled per minute [1]. Assembly of ribosomes requires ribosomal RNA transcribed by RNA Polymerase (Pol) I, which accounts for > 60% of total RNA content in a rapidly growing cell [2], as well as the RNA Pol III-transcribed 5S rRNA. Moreover, ribosomal protein (RP) gene expression accounts for >50% of the mRNAs synthesized by RNA Pol II, which also transcribes the > 200 ribosome biogenesis (RiBi) genes encoding the processing factors involved in ribosome biogenesis [1,3]. Thus, for such an active process to be precise, coordination between all the events involved in synthesis of its components is required. Key to this coordination is mTORC1 (mechanistic Target of Rapamycin Complex 1), a major regulator of growth and proliferation, which is active under nutrient sufficient conditions or in the absence of any cellular stress such as that caused by DNA damage or environmental signals [4–6].

In this review, we will focus on RP gene regulation in yeast, mainly *S. cerevisiae*, which is the primary model system in which such regulation has been investigated. Computational analyses have pointed to a shared ancestral origin of two key transcription factors with inferred activator and repressor functions, respectively. We discuss the correlation between RP gene duplication and retention of both transcription factor paralogs, and we consider the possibility that both transcription factors function as activators.

2. mTORC1 controls cell growth and proliferation

mTORC1 contains the Ser/Thr protein kinase Tor, which is functionally conserved between all eukaryotes, from *S. cerevisiae* to *Homo sapiens* [7]. In budding yeast, mTORC1 may be assembled with Tor1p or Tor2p, although Tor1p is preferred (as evidenced by limited copurification of Tor2p and dedicated mTORC1 subunits) [8,9]. Additional mTORC1 components include Kog1p (Kontroller Of Growth; the homolog of mammalian Raptor), Lst8p (Lethal with Sec Thirteen; the homolog of mLST8), and Tco89p (Tor Complex One; for which there is no mammalian homolog) [10]. In contrast, Metazoans encode a single Tor protein (named mammalian Tor or mTor) [11]. All eukaryotes have two functional TOR complexes, mTORC1 and mTORC2. In higher eukaryotes, mTor is the kinase component of both TORC1 and TORC2 complexes in combination with different subunits. In budding yeast, mTORC2 contains Tor2p with its required subunits [9], and *TOR2* is essential because mTORC2 is required for maintaining cell structure through actin polarization [12].

Yeast mTORC1 is maintained in an active state during amino acid sufficiency, while metazoan mTORC1 additionally responds to growth factors and mitogens (Fig. 1) [4,5]. Upstream activators such as amino acids target cytoplasmic mTORC1 localized to the lysosomal surface (the vacuole in yeast) [13], and this results in mTORC1-mediated phosphorylation of downstream targets such as regulators of translation [14–16]. Conversely, mTORC1 is inactivated as a result of stress and nutrient insufficiency [9,17]. mTORC1 thereby mediates a switch between active growth and the temporary growth cessation, which is imposed by starvation or required to prioritize recovery from DNA damage or stress. When active, mTORC1 signals to downstream effectors responsible for managing cell size and progression of the cell cycle, and it stimulates several transcriptional networks responsible for anabolic processes while simultaneously inhibiting catabolic pathways such as autophagy. In contrast, inhibition of mTORC1 promotes the catabolic state associated with starvation to allow energy to be dedicated to critical functions and survival [5,8,9,18–21].

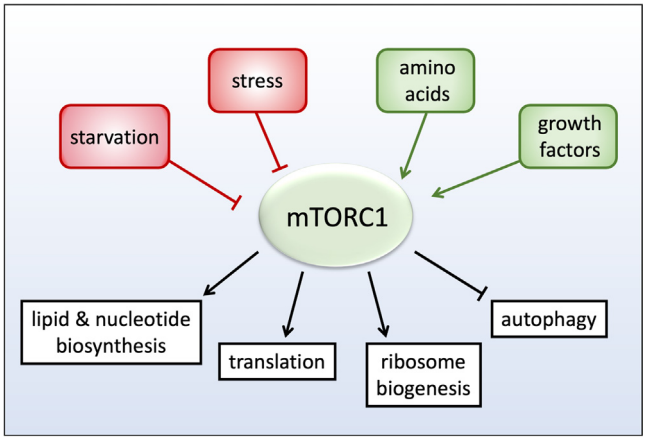

Fig. 1. Effectors and targets of mTORC1. An overview of events, which activate (green arrows) or inhibit (red lines) mTORC1. Examples of mTORC1 targets shown below. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The mTORC1 complex is characterized by its acute inhibition by the immunosuppressant rapamycin (sirolimus) [8]. Rapamycin binds FKBP (FK506 binding protein), and the complex then directly binds and inhibits mTORC1 (and indirectly inhibits mTORC2 by a mechanism thought to involve compromised complex assembly, provided chronic exposure to sufficient concentrations of this compound) [22–24]. Yeast FKBP12, also known as Fpr1p (FK506-sensitive proline rotamase), in addition functions as a transcription factor for most RP genes [25]. However, yeast strains lacking *FPR1* have no obvious phenotype, except that they are insensitive to rapamycin and FK506 [8,25].

2.1. Nuclear functions of mTORC1

mTORC1 also has nuclear functions under nutrient replete conditions, where its main targets include genes encoding proteins involved in ribosome biogenesis (Fig. 2). In both yeast and mammalian cells, Tor1p/mTor binds the promoters of both Pol I- and Pol III-transcribed rRNA genes, whereas rapamycin treatment or nutrient deprivation causes Tor1p/mTor to leave the nucleus, conditions under which transcription is repressed. In addition, RNA Pol III transcription of 5S rDNA is negatively controlled by Maf1p, and this negative regulation is alleviated when Maf1p is phosphorylated by active mTORC1 [26–31].

RNA Pol II is responsible for transcription of RP and RiBi genes. RPs are integral to the ribosome, whereas RiBi genes encode factors, which do not become part of the mature ribosome, but are required for its assembly. Examples include factors required for rRNA modification and ribosome assembly as well as enzymes involved in nucleotide metabolism and translation [32]. RiBi genes have been primarily characterized by the presence of promoter ele-

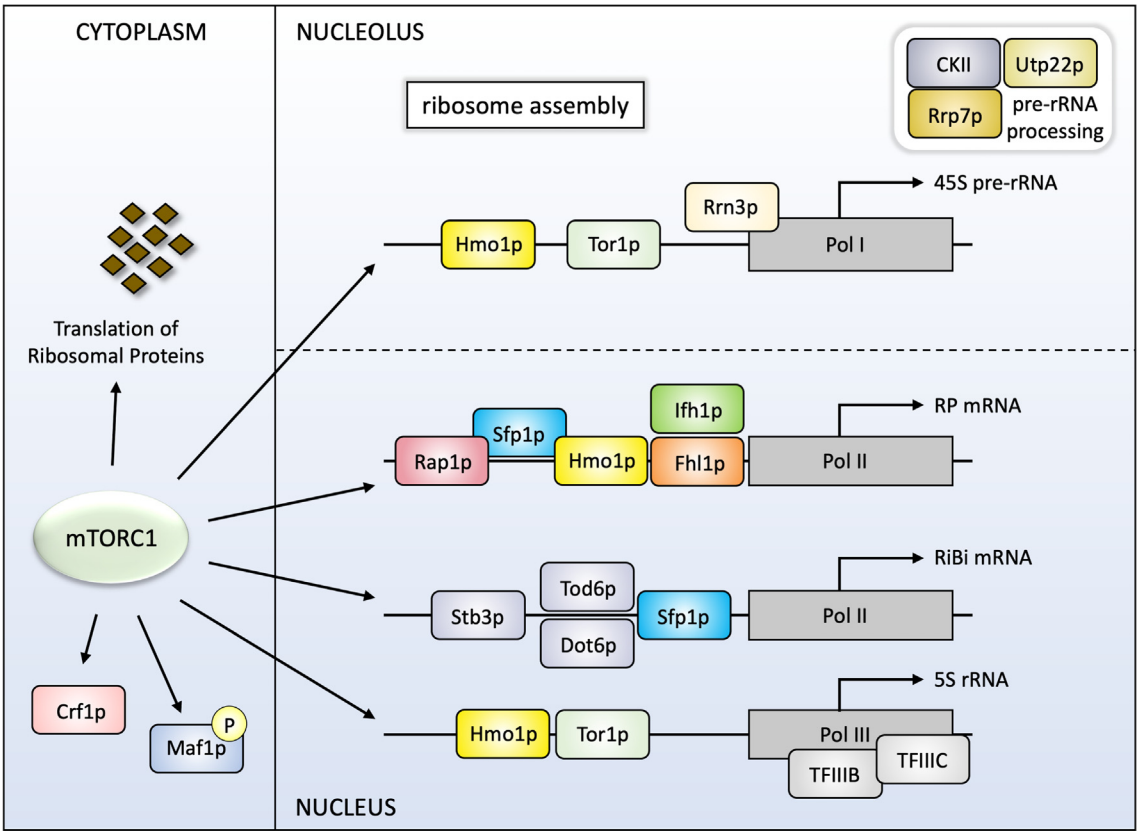

Fig. 2. mTORC1 functions to control ribosome biogenesis. In the cytoplasm, active mTORC1 generally promotes translation, for example by direct phosphorylation of 4E-BP. Newly synthesized RPs translocate to the nucleolus for ribosome assembly. mTORC1 indirectly (*via* PKA and Yak1p) controls the subcellular localization of Crf1p, and it phosphorylates Maf1p to prevent its nuclear localization, thereby ensuring active Pol III transcription. Nuclear mTORC1 functions include direct binding of Tor kinase to both Pol I- and Pol III-transcribed rRNA genes and control of transcription factors associated with transcription by Pols I, II, and III. Inhibition of mTORC1 causes release of Ifh1p from RP genes and its sequestration within the nucleolar CURI complex, a process that also sequesters the RiBi proteins Utp22p and Rrp7p.

ments that recruit transcriptional repressors such as Dot6p and Tod6p, which in turn assemble the Rpd3L histone deacetylase to downregulate transcription during stress [33]. Thus, RiBi gene promoters were originally characterized by the presence of repressive promoter elements. More recently, RiBi gene promoters have also been reported to bind General Regulatory Factors (GRFs), which participate both in maintaining robust expression in rich media and in mediating reduced expression in response to nutrient limitation [34]. RP genes have distinct promoter architectures, and they have been sub-categorized based on specific promoter elements and the assortment of transcription factors that bind to these genes, as discussed in more detail below.

Notably, many of these transcription factors are regulated by mTORC1 [34–38]. Thus, mTORC1 controls ribosome biogenesis by coordinately controlling RNA Pol I-, II-, and III- transcribed genes. A lack of coordination causes a disturbance in ribosome assembly, leading to physiological conditions in humans categorized as ribosomopathies, one consequence of which is proteotoxic stress [39,40]. Patients with ribosomopathies generally present with tissue-specific abnormalities. One example is the congenital bone marrow failure syndrome known as Diamond-Blackfan anemia, which is most often characterized by mutations in specific RPs. In addition, many ribosomopathy patients are at greater risk of developing cancers, either because mis-assembled ribosomes may favor translation of oncogenic products, because of affected RPs moonlighting in other contexts, or due to general effects on cellular metabolism (for a recent review, see [41]). As a central regulator of cell proliferation, mTORC1 has been linked to several human cancers, as mutations in its upstream activators lead to hyper-activation. Such mTORC1 hyper-activation in turn supports the proliferative needs of tumor cells [42].

3. RP gene regulation in yeast

Regulation of RP gene transcription is key to maintaining the proper stoichiometry and assembly of ribosomal components. RPs are functionally conserved, and their numbers are also fairly constant between the three domains of life. In comparison to prokaryotes, which have 55 RPs, eukaryotes have 78–80. However, the total number of genes coding for these proteins varies between different kingdoms. Among eukaryotes, fungi have 138 RP genes, plants have about 217 RP genes, and animals have 79 RP genes, in all cases encoding 79 RPs, while protists transcribe 79 RP genes coding for 78 RPs [43,44]. In addition, yeast Asc1p and its mammalian homolog RACK1 (receptor for activated C-kinase 1) are components of the 40S ribosomal subunit, which link signal transduction pathways to the ribosome [45,46]. Although genome sizes of fungi and animals vary greatly, the number of RP genes is greater in fungi despite their overall smaller genomes. Thus, the number of RP genes is independent of the genome size and rather show a directly proportional relationship with the occurrence of genome duplication events [43,47,48].

A whole genome duplication (WGD) event occurred in a common ancestor of several yeast genera. The WGD encompassed the entire genome of the precursor, as for example evidenced by the organization of the 16 centromeres of *S. cerevisiae* into eight pairs, each syntenic with non-WGD yeast species, implying that the ancestor had eight chromosomes and that the WGD event gave rise

to a 16-chromosome descendant [49–51]. While many duplicated genes were subsequently lost, the majority of duplicated RP gene pairs (ohnologs) were retained in *S. cerevisiae*, with only 19 of 138 RP genes being unique. Of the duplicated genes, only 22 encode identical proteins. The duplicated RP genes are located on different chromosomes, their expression patterns have diverged, and not all duplicated RPs are functionally interchangeable [44,52,53]. As a corollary, ribosome activity may be regulated based on changes in composition and for instance optimized for translation of certain mRNAs or modified in response to stress [54,55]. This vast number of RP genes must be expressed in a coordinated fashion yet allowing for preferential expression of specific genes in response to environmental cues. Unlike the situation in bacteria, where RP genes are organized in operons, eukaryotic RP genes are expressed individually and even feature variable promoter architecture, rendering their regulation much more complex [35,37,44,56,57]. In addition to transcriptional control, it has been reported that the presence of introns in RP genes, which encode the majority of spliced introns in yeast, mediates repression in response to starvation in an mTORC1-dependent manner [58].

3.1. Three categories of yeast RP genes

RP genes in *S. cerevisiae* feature regulatory motifs, which are required for gene specific expression control. RP genes are classified into three categories based on the transcription factors associated with them. Most of the upstream activating sequences (UAS) in yeast RP genes are associated with Rap1p (repressor/activator protein) as seen in categories I (69 genes) and II (60 genes) [35]. Rap1p association enables the recruitment of Forkhead-like 1 (Fhl1p), which in turn recruits the activator Interacts with Fhl1p (Ifh1p) [35,59,60]. The two Rap1p-associated categories of RP genes are further sub-divided on the basis of presence or absence of the high mobility group (HMGB) protein Hmo1p [61]. Category I RP genes localize Hmo1p on their promoters, where it is required for binding of Fhl1p, while category II is devoid of Hmo1p [35,37,62,63]. Category III RP genes are independent of Rap1p, but they require ARS binding factor 1 (Abf1p) [35,37,63]. Further, RP gene promoters have also been shown to bind the nutrient- and stress-sensitive split finger protein (Sfp1p) and Fpr1p. For category I and II promoters, Sfp1p binding depends on Ifh1p, while other promoters, including those of RiBi genes, are characterized by binding of Sfp1p directly to chromatin at a gAAAAWTTTTc motif (with lower case letters indicating a less conserved position and W representing A or T) [25,37,64–67].

3.2. The role of Hmo1p

S. cerevisiae Hmo1p has been shown to communicate mTORC1 signaling to downstream target genes [62,63,68–73]. Genome-wide, Hmo1p binding to target sites is variable, with particularly enhanced binding to Pol I-transcribed rDNA, category I RP genes, and its own promoter [62]. On these genes, absence of Hmo1p results in an attenuated response to mTORC1 inhibition, and another consistent feature is that Hmo1p is released during prolonged mTORC1 inhibition [62,70,72,74]. The function of Hmo1p at these genes differs. On rDNA, Hmo1p promotes transcription, perhaps in part by limiting nucleosome occupancy [74,75]. On Category I RP genes and on the *HMO1* promoter, Hmo1p binding facilitates binding of Fhl1p (and in turn Ifh1p) as evidenced by reduced binding of Fhl1p in *hmo*1Δ yeast strains. Conversely, absence of Fhl1p reduces occupancy of Hmo1p on target genes [35,62,69,71]. While deletion of *IFH1* is lethal, and deletion of *FHL1* confers a severe growth defect, transcription of RP genes is not markedly affected by absence of Hmo1p, despite reduced Fhl1p/Ifh1p complex assembly [62,70,76,77]. It was recently shown that Fpr1p likewise promotes Fhl1p binding to certain RP genes, and it is therefore likely that the synthetic lethality or synthetic growth defect (depending on strain background) associated with deletion of both *FPR1* and *HMO1* may relate to failure to recruit Fhl1p to target genes, specifically *RPL25*, which is markedly downregulated in *hmo1Δfpr1Δ* cells [25,70,78]. On RP genes, deletion of *HMO1* does impair recruitment of transcription factor TFIID, however, Hmo1p may facilitate accurate start site selection, likely by masking a nucleosome-free region and preventing non-productive preinitiation complex assembly [79,80].

3.3. The proposed roles of dedicated transcription factors Ifh1p and Crf1p

As alluded to above (Section 3.2), the genetic background used may affect the outcome of gene deletions. An *hmo*1Δ*fpr*1Δ strain was reported to be inviable in the Y388 strain background [81], whereas the *hmo*1Δ*fpr*1Δ double deletion confers a synthetic growth phenotype in S288C cells [25,78]. The basis for this difference is unknown, but it could possibly relate to relative expression levels of *RPL25*. S288C is a common laboratory strain, produced by a deliberate sequence of crosses to optimize its use for analysis of biochemical mutants, and it also serves as the reference strain [82]. However, S288C is missing a number of genes present in other laboratory strains. Strain W303 was derived from S288C by introduction of several markers, hence it is a particularly popular laboratory strain, but ~ 15% of its genetic material is from other sources, and it has distinct phenotypes [83]. Another relevant strain, which contains some genetic material from S288C is JK9-3d (and its derivative TB50); TB50 was used for early experiments pertaining to Ifh1p and Crf1p function [59]. As specified further below (Section 3.5), a direct comparison of experiments performed with different yeast strains should consider the possibility of strain background substantially affecting experimental outcomes.

Regulation of the majority, if not all of yeast RP genes depends on Ifh1p, which binds actively expressing Category I and II RP genes and a few RiBi genes, as determined by Chromatin Immunoprecipitation (ChIP). While inhibition of mTORC1 has little effect on Fhl1p occupancy, Ifh1p dissociates rapidly upon exposure to stress [59,60,63,76,84–87]. Ifh1p has also been proposed to play a role on Category III RP genes, as it may be detected on these genes by the more sensitive Chromatin Endogenous Cleavage (ChEC) approach in which the protein of interest is fused to micrococcal nuclease [37]. Notably, anchor-away experiments have shown that depletion of Ifh1p from the nucleus is sufficient to repress transcription [37]. Anchor-away is a technique by which the function of essential (or near-essential) nuclear proteins may be assessed, and it involves the conditional tethering of the target protein to an abundant cytoplasmic protein, thereby depleting the target from the nucleus [88].

As noted above, deletion of *IFH1* is lethal, consistent with a key role for Ifh1p in activating RP gene activity. However, the double deletion of *IFH1* and *FHL1* is viable (albeit sick), indicating that the activator function of Ifh1p is essential only in cells expressing Fhl1p [77,89]. The original interpretation of this observation was that Fhl1p functions as a repressor in absence of Ifh1p [77]. This interpretation would also be consistent with the observation that both Hmo1p and Fpr1p promote Fhl1p binding (and in turn Ifh1p), yet the absence of either Hmo1p or Fpr1p has little effect on RP gene transcription, perhaps because of reduced binding of the repressor, Fhl1p, and in turn reduced dependence on the activator, Ifh1p [25,62]. On the contrary, absence of Hmo1p leads to increased activity of the *HMO1* promoter, but only when the so-called IFHL site is present; this site was previously shown to correlate with binding of Fhl1p and Ifh1p to RP genes [60,71].

Several mechanisms have been proposed to explain how mTORC1 activity is communicated to RP genes. One response is based on the subcellular localization of protein kinase A (PKA) and the PKA-regulated kinase Yak1p. Using TB50 yeast cells, the TOR-PKA pathway was suggested to regulate RP gene transcription in a Fhl1p-dependent process, by controlling assembly of either of the two co-factors Ifh1p or Co-repressor with Fhl1p (Crf1p) [59]. According to this model, Crf1p is primarily cytoplasmic when mTORC1 is active, whereas inactivation of mTORC1 leads to activation of Yak1p, which phosphorylates Crf1p to facilitate its nuclear import. Once in the nucleus, Crf1p competes with Ifh1p for binding to Fhl1p and represses transcription of RP genes [59]. As detailed below, this model appears to be strain-specific and unlikely to apply generally, and it is difficult to reconcile with more recent reports of Crf1p functioning as an activator.

Ifh1p shows a dynamic response to environmental stress or nutrition deprivation, regardless of strain background, as communicated by mTORC1 inhibition [59,60,63,76,84–87]. According to one mechanism for removing Ifh1p from RP genes, which was illustrated using the W303 strain background, Ifh1p dissociates from RP gene promoters and associates with the UTP-C 90S pre-ribosome complex localized in the nucleolus. The UTP-C 90S pre-ribosome complex is involved in processing pre-ribosomal RNA, and it is a multi-subunit protein complex containing Casein Kinase 2 (CK2) and two RiBi proteins, U three protein-22 (Utp22p) and Ribosomal RNA Processing-7 (Rrp7p). These proteins form the CURI complex upon association with Ifh1p (with CURI named for the components CK2, Utp22p, Rrp7p, Ifh1p), and this complex formation is crucial for titrating away Ifh1p from RP genes while simultaneously sequestering proteins required for pre-rRNA processing [90–93]. The rapid removal of Ifh1p has also been suggested to depend on its entrapment within aggregates of RP proteins, which accumulate as a consequence of ribosome assembly stress [85]. This expression control could possibly be reinforced by activation of Yak1p on mTORC1 inhibition, by phosphorylating Crf1p and enabling its movement from the cytoplasm to the nucleus and binding target gene promoters abandoned by Ifh1p.

Inhibition of mTORC1 also affects post-translational modifications of Ifh1p. For example, the histone acetyl transferase Gcn5 has been implicated in acetylating Ifh1p, particularly in its N-terminal domain [94,95]. Cellular stress, as imposed for example by addition of rapamycin, results in reduced levels of acetylation, and acetylation is increased upon recovery from stress. One notable consequence of reduced Ifh1p acetylation appears to be reduced protein stability [95]. Interestingly, hypoacetylated, promoter-bound Ifh1p was inferred to support a rapid increase in RP gene transcription on recovery from stress or starvation, whereas acetylation of Ifh1p appears to limit this initial burst of activity [94].

3.4. The evolutionary relationship between Ifh1p and Crf1p

IFH1 and *CRF1* appear to be the result of an ancient WGD event [47]. This gene pair evolved from a common ancestor, which is still found, for example, in *Kluyveromyces lactis,* a pre-WGD relative of *S. cerevisiae* (Fig. 3A). While pre-WGD orthologs are similar to Ifh1p, Crf1p lacks the acidic N-terminal *trans*-activation domain, and it features a greater rate of amino acid substitutions compared to Ifh1p (Fig. 3B). These observations suggest that Ifh1p represents the pre-WGD ancestor, whereas the function of Crf1p was derived following the WGD event [47]. Based on the previously suggested activator and repressor functions of *S. cerevisiae* Ifh1p and Crf1p, respectively [59], the Ifh1p/Crf1p ancestor was proposed to have both activator and repressor functions as evidenced by conserved and coordinated repression of RP gene expression during stress in pre-WGD species such as *K. lactis* [47].

Ifh1p and Crf1p were not retained in all post-WGD species, rather the *Candida glabrata* genome reflects a loss of Crf1p [47,96]. Two possible theories could explain the evolution of Crf1p; with two functional copies of the ancestral protein, each of which experiencing mutations, an alteration in a functional domain in one of the copies may have been backed by the paralog to retain the overall functionality, ultimately leading to specialized functions of each paralog [97,98]. Alternatively, managing the

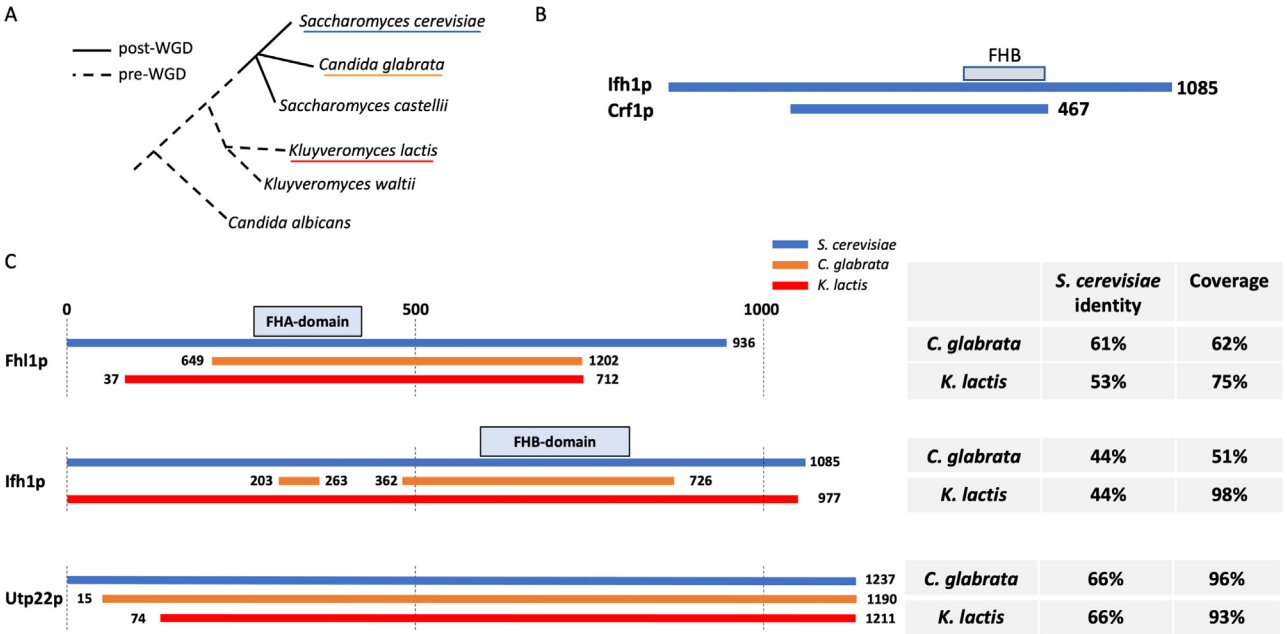

Fig. 3. Conservation of transcription factors. A. Phylogenetic tree of select ascomycetes. Species identified with solid lines diverged after the WGD event, and species identified with dashed lines diverged prior to the WGD. Adapted from [47]. B. *S. cerevisiae* Ifh1p and Crf1p. Both proteins feature a conserved forkhead-binding (FHB) domain. Crf1p lacks N- and C-terminal extensions compared to Ifh1p. C. Alignment of Fhl1p, Ifh1p, and Utp22p from *S. cerevisiae* (blue), *C. glabrata* (orange), and *K. lactis* (red) using NCBI Blastp. Identity and coverage relative to the corresponding *S. cerevisiae* ortholog is indicated at the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increased dosage of RP gene products post duplication may have been favored by the evolution of dedicated regulators [99,100]. *C. glabrata*, which lost Crf1p, has 85 RP genes, which is comparable to the pre-WGD relatives, e.g., *K. lactis* (81 RP genes) and *C. albicans* (84 RP genes). By contrast, *S. cerevisiae* and *S. castellii*, in which Crf1p is retained, have 138 RP genes. One interpretation is that the need for a specialized regulator is related to the requirement for more intricate transcription control in strains with a higher number of RP genes. For example, recent analyses of duplicated gene pairs in mammalian genomes suggested that tandem duplicates tend to be co-regulated, unlike duplicate genes located on separate chromosomes [101]. As noted above, duplicated RP genes in *Saccharomyces* are located on different chromosomes, and compared to single copy RP genes, duplicate RP genes in *S. cerevisiae* are expressed at lower levels [102].

3.5. Is Crf1p really a repressor?

Ifh1p and Crf1p have a conserved forkhead-binding (FHB) domain (Fig. 3B), which binds the forkhead-associated (FHA) domain in Fhl1p [59], and phosphorylation of the FHB domains by CK2 is required for the interaction with the FHA domain of Fhl1p [91]. Structurally, both Ifh1p and Crf1p remain uncharacterized, and secondary structure predictions indicate extended regions of disorder. Such disorder is not uncommon in eukaryotic transcription factors, and it may confer a plasticity required for interaction with multiple cellular targets [103]. The N-terminal domain of Ifh1p was shown to be required for the removal of Ifh1p from RP genes on inhibition of mTORC1, and Crf1p also lacks the C-terminal region, which in Ifh1p is required for its interaction with Utp22p and sequestration into the CURI complex [90]. These features could explain why Ifh1p is key to the mTORC1-sensitive regulation of RP gene expression.

While these characteristics may explain how the activator Ifh1p responds to mTORC1 inhibition, they do not shed much light on the function of Crf1p, beyond documenting molecular mechanisms underlying its binding to RP gene promoters and Fhl1p. That Ifh1p and Crf1p have distinct functions is indubitable and it is supported by several additional lines of evidence, including the expression pattern of *IFH1* and *CRF1* on mTORC1 inhibition. In *S. cerevisiae* strains W303 and BY4741, rapamycin treatment and amino acid limitation induces *CRF1* expression with a simultaneous reduction in *IFH1* expression, and a similar expression pattern was reported in *S. castellii*, another post-WGD strain, which encodes Crf1p [47,104]. And while deletion of *IFH1* is lethal, deletion of *CRF1* is not [77]. As noted above, Crf1p was reported to be required for mTORC1-mediated repression of RP gene expression to be manifest in *S. cerevisiae* strain TB50 [59]; in apparent support of a repressor function for Crf1p, *C. glabrata*, which has lost Crf1p, does not experience concerted downregulation of RP genes in response to stress [47].

Perhaps more importantly, whether Crf1p truly functions as a repressor appears to be in question. Notably, the requirement for Crf1p is not conserved among *S. cerevisiae* strains. The inference that Crf1p functions as a repressor in controlling of RP gene expression was based on experiments with *S. cerevisiae* strain TB50; by contrast, RP gene expression declines in strain W303 after mTORC1 inhibition irrespective of the presence of Crf1p [37,90,91,105,106]. These differences were inferred to derive from multiple differences, as opposed to a single gene, as suggested by the behavior of haploid segregants resulting from crosses between W303 and TB50 [106]. It has also been reported that plasmid-based overexpression of Crf1p in an *ifh*1Δ strain (W303 genetic background) reverses the lethality of the *IFH1* deletion, suggesting that Crf1p may substitute for Ifh1p in enabling RP gene expression. This experiment also showed that repression of Category I and II RP genes in response to rapamycin treatment is compromised when Crf1p entirely replaces Ifh1p [37], consistent with absence of the N- and C-terminal protein domains in Crf1p, which have been implicated in removing Ifhl1p from RP gene promoters. Furthermore, we have recently reported that endogenous Crf1p also promotes expression of select Category I and II RP genes during balanced growth as evidenced by reduced mRNA accumulation in a *crf*1Δ strain [105]. Taken together, these observations suggest that Crf1p functions as an activator.

Ifh1p and Crf1p bind the same genes, and they function in overlapping conditions, features generally proposed to imply at least partial redundancy for duplicated transcription factors. A relevant comparison is the ancestral transcription factor Msn, which gave rise to *S. cerevisiae* Msn2 and Msn4 following the WGD event. Msn2 and Msn4 regulate the same stress response genes, and it has been proposed that they confer adaptive benefit, not through functional divergence, but by cooperation [107]. In this scenario, the advantage to having two separate factors is that stress responsiveness is optimized while adverse effects on cell growth are attenuated. The possibility that Ifh1p and Crf1p cooperate in activating RP genes is therefore not unprecedented.

3.6. An alternate explanation for the strain-specific requirements for Crf1p

In *C. glabrata*, which has lost Crf1p, RP gene expression is not suppressed during stress [47]. This phenotype is similar to that characteristic of *S. cerevisiae* TB50 deleted for *CRF1* [59,91]. These observations speak to a role for Crf1p in communicating mTORC1 inhibition to target genes. However, what would be the likelihood of the repressor function of Crf1p to be entirely lost in the closely related *S. cerevisiae* strain W303? First of all, that *CRF1* is upregulated during stress in this strain would be consistent with a specific function under such conditions [47,104]. Secondly, according to the *Saccharomyces* Genome Database [108], the amino acid sequences of Crf1p, Fhl1p, and Hmo1p are invariant between *S. cerevisiae* strain W303, in which Crf1p is dispensable for RP gene regulation during stress, and JK9-3d, the parent strain of TB50, in which Crf1p is required to maintain the repressed state. This suggests that strain-specific differences are unrelated to Crf1p *per se* or to its interaction with Fhl1p. Differences between W303 and TB50 in terms of the dependence on Crf1p for communication of mTORC1 inhibition were inferred to be due to multiple changes [106], also suggesting that they are not merely due to loss of a specific Crf1p function in W303.

Ifh1p is an essential activator of RP genes, and its removal from gene promoters in response to mTORC1 inhibition depends on its interaction with Utp22p, which in turn leads to the sequestration of both proteins in the CURI complex [90]. These experiments, which were performed with *S. cerevisiae* W303, show that in wild-type cells, Ifh1p leaves the promoter 5 min after rapamycin treatment, and Ifh1p occupancy remains low after 25 min. Notably, depletion of Utp22p does not change the rapid dissociation of Ifh1p, but it results in faster re-binding of Ifh1p to RP gene promoters, with readily detectable binding 10 min after addition of rapamycin [90]. A possible explanation for the strain-specific dependence on Crf1p is therefore that the dynamics of CURI complex assembly/disassembly varies between strains (Fig. 4). According to this model, Crf1p may be dispensable for RP gene regulation in W303 because Ifh1p remains stably sequestered in the CURI complex or within RP aggregates, causing its re-binding to RP gene promoters to be slow. This model also predicts that Ifh1p re-binding following rapamycin treatment would be faster in *S. cerevisiae* TB50 (and *C. glabrata*), unless Crf1p can take its place. According to this prediction, Ifh1p should quickly (<5 min) leave RP gene promoters on rapamycin treatment of TB50 cells, but it

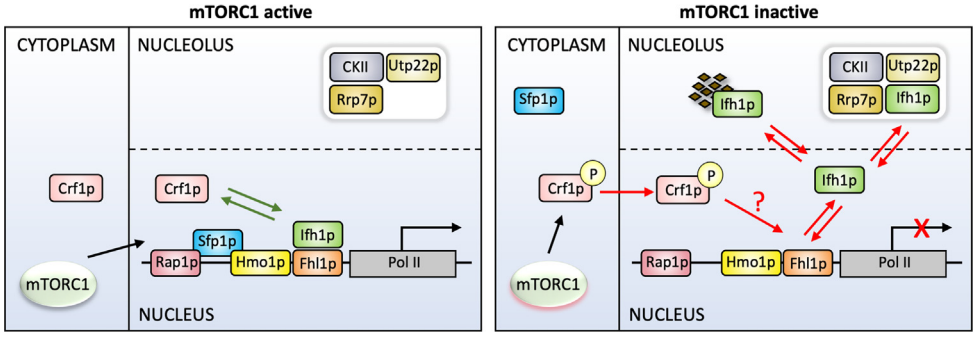

Fig. 4. The dynamics of CURI complex formation may dictate dependence on Crf1p. When mTORC1 is active (left), Crf1p is predominantly cytoplasmic. Nuclear Crf1p may bind Fhl1p and promote transcription of RP genes if Ifh1p is limiting. Direct phosphorylation of Sfp1p by mTORC1 ensures its nuclear localization. The nucleolar UTP-C subcomplex composed of CKII, Utp22p, and Rrp7p processes pre-rRNA. On inhibition of mTORC1 (right), Sfp1p leaves the nucleus. Crf1p is phosphorylated and translocates to the nucleus. Ifh1p interacts directly with Utp22p, and both proteins become sequestered in the CURI complex. Alternatively, Ifh1p may become trapped within newly synthesized RP aggregates. Absence of Ifh1p is sufficient for repression of RP gene transcription. We propose that Crf1p may be required to maintain reduced RP gene expression under conditions where sequestration of Ifh1p is transient and re-binding of Ifh1p to RP genes is rapid.

should become detectable again within 30 min in cells deleted for Crf1p. This model does not necessarily imply that Crf1p functions as a repressor in the traditional sense, only that Crf1p prevents binding of a better activator, and it is consistent with more recent evidence that Crf1p functions to activate transcription.

The amino acid sequence of Ifh1p from *S. cerevisiae* W303 and TB50 varies at three positions, two of which within the FHB domain, which has not been implicated in the dynamics of Ifh1p removal from gene promoters. However, position 991, which is within the C-terminal region required for interaction with Utp22p, is a proline in the W303-encoded homolog and a serine in the protein encoded by TB50. While Rrp7p and the CK2 subunits from W303 and TB50 are identical, the Utp22p homologs differ at one position, however, it is not known which part of Utp22 is involved in Ifh1p interaction. Nonetheless, it is conceivable that these amino acid changes collectively alter the interaction between these proteins. By contrast, the *C. glabrata* Utp22p homolog shares only 66% identity with *S. cerevisiae* Utp22p, and its Ifh1p homolog is quite divergent and does not share any sequence conservation with *S. cerevisiae* Ifh1p at the N- and C-termini (Fig. 3C). Accordingly, it is plausible that the Utp22p-dependent removal of Ifh1p in response to mTORC1 inhibition is not conserved in *C. glabrata*, accounting for the lack of RP gene regulation during stress in this yeast species.

4. A role for Ifh1p and Crf1p at non-RP genes

Amongst the very few non-RP gene targets of Fhl1p-Ifh1p are *HMO1, UTP22* and *RRP7*. Fhl1p, Ifh1p, and Hmo1p bind these gene promoters under nutrient-replete conditions [35,60,87,90,105]. Addition of rapamycin represses transcription, and it causes the release of Ifh1p, similar to what occurs on RP genes, and the dissociation of Ifh1p is followed by accumulation of Crf1p. In the W303-derived *crf*1Δ strain, *UTP22* and *RRP7* mRNA abundance decreases on mTORC1 inhibition, indicating that Crf1p is dispensable for regulation [105]. While this observation may seem unremarkable in that it recapitulates the regulation seen for RP genes, it is notable because it reveals a regulatory mechanism, which is unlike that reported for other RiBi genes. Utp22p and Rrp7p not only serve a RiBi function in pre-rRNA processing, but they are also key to the control of RP genes, which rationalizes why *UTP22* and *RRP7* may be regulated by a mechanism more akin to that of the Hmo1p-dependent Category I RP genes.

Regulation of *HMO1* appears to be more complex. Rapamycin treatment does not lead to reduced *HMO1* mRNA abundance in a W303-derived *crf*1Δ strain, except under conditions of prolonged (>1h) exposure [105]. It is also notable that *HMO1* mRNA abundance is not reduced as efficiently as RP genes (<50%) when wild-type cells are treated with rapamycin. A possible explanation for these observations is that Ifh1p can re-associate faster with the *HMO1* promoter as compared to RP gene promoters, particularly when Crf1p is absent.

One notable distinction between *HMO1* and RP genes was revealed by the *tor*1Δ phenotype; in absence of Tor1p, inhibition of mTORC1 does not lead to reduced *HMO1* expression whereas repression of RP genes is unaffected, as is that of *UTP22* and *RRP7*. This difference is likely related to another distinction, which is that Tor1p binds directly to the *HMO1* promoter, but not to the promoters of RP genes, *UTP22*, or *RRP7* [31,87,105]. This indicates that when mTORC1 is assembled with Tor2p, RP genes and *UTP22* and *RRP7* remain responsive to rapamycin, whereas *HMO1* does not. We therefore suggest that the expression of the *HMO1* gene is less responsive to mTORC1 inhibition on account of the direct promoter binding of the mTORC1 complex, perhaps because it maintains a phosphorylation state of Ifh1p, which promotes faster reassociation.

5. Evolution of promoter elements

As outlined above (Section 3.1), RP gene promoters have been subdivided into three categories, each of which characterized by the binding of a specific set of transcription factors. The presence of conserved promoter elements in RP genes was appreciated decades ago, with the description of the HOMOL1-box and RPG-box elements [109,110]. This inspired interest in understanding how specific promoter elements participate in the control of gene expression, particularly in strains with duplicated RP genes for which dosage compensation is required. For instance, consensus Rap1p binding sites are frequently occurring in duplicate, approximately 200–500 bp upstream of the transcriptional start of Category I and II RP genes, and the IFHL site was reported to correlate with Fhl1p/Ifh1p binding [60,111,112]. By contrast, Category III RP genes feature a distinct promoter architecture and the binding of the general transcription factor Abf1p in place of Rap1p. Such dependence on different transcription factors permits varied responses to different growth conditions [37].

The rewiring of gene regulatory networks can lead to different expression patterns. One scenario, which appears to characterize RP genes, comprises a generalized transcription factor, such as Rap1p or Abf1p, which in turn associates with different combinations of specialized factors to provide an adaptive response. Even for paralogous transcription factors, regulatory sequences may

have diverged due to either drift or selection, and the resulting promoters may then operate optimally under different conditions, building in a redundancy to ensure adequate production of the gene products [37]. Indeed, a comparison of paralogous RP gene pairs reveals non-identical expression patterns, rationalizing the retention of duplicated genes [53].

This type of promoter evolution described above has also occurred between pre- and post-WGD species of yeast. For example, IFHL elements and Rap1p binding sites are over-represented in RP gene promoters in both pre- and post-WGD yeast species, consistent with a regulatory mechanism that depends on Ifh1p. *C. glabrata* RP genes feature similar promoter elements as *S. cerevisiae* RP genes, except for the additional presence of an MCM1 element. However, the MCM1 site is also found in RP genes from the pre-WGD species *K. lactis*, whose RP genes are repressed during stress. These observations support the interpretation that lack of RP gene repression in *C. glabrata* is due to evolutionary changes in transcription factors, not *cis*-elements [47].

6. Summary and outlook

Control of ribosome biogenesis is paramount to cellular health, and the mTORC1 pathway is key to this process. Perturbations generate ribosome assembly stress, which in turn provokes a range of pathological conditions, underscoring the necessity of a complete molecular understanding of underlying mechanisms. One component of this process is the transcriptional control of RP genes. Much insight into this process has been derived from experimental analyses of gene regulation in the model yeast *S. cerevisiae*, combined with computational analyses of related yeast species. Recent evidence has emphasized the dynamics of Ifh1p removal from RP genes in response to mTORC1 inhibition as key to gene regulation, either by the sequestration of Ifh1p in the CURI complex or in aggregates of newly synthesized, unassembled RPs, which accumulate as a consequence of the ribosome assembly stress response (RASTR) [85,90]. Much remains to be deciphered about these processes, including mechanisms leading to protein aggregation.

Ifh1p and its paralog Crf1p derive from a common ancestor [47]. We propose that the ancestral mode of RP gene regulation during stress involved the removal of the Ifh1p/Crf1p ancestral activator, possibly by a mechanism akin to the CURI or RASTR complex formation described for *S. cerevisiae*. We further suggest that Crf1p serves the same function in all *S. cerevisiae* and possibly all post-WGD yeast strains in which this paralog was retained. It functions as a backup activator in situations where Ifh1p is in short supply, perhaps to ensure a baseline level of RP gene expression that may increase rapidly when cells are released from mTORC1 inhibition. Such backup activation may be particularly pertinent when RP genes are duplicated, a situation that may more readily cause Ifh1p to become limiting. When mTORC1 is active, Crf1p is primarily cytoplasmic. This subcellular localization makes sense because Crf1p is a constitutive activator, which is not removed from RP genes in response to mTORC1 inhibition. Accordingly, nuclear levels are lower in rapidly growing cells to prevent it from competing too effectively with Ifh1p for binding to target promoters. This model for Crf1p function consolidates both available experimental evidence as well as computational predictions.

CRediT authorship contribution statement

Sanjay Kumar: Writing – original draft, Writing – review & editing, Visualization. **Muneera Mashkoor:** Writing – original draft, Writing – review & editing. **Anne Grove:** Writing – original draft, Writing – review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Warner JR. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 1999;24(11):437–40.
[2] von der Haar T. A quantitative estimation of the global translational activity in logarithmically growing yeast cells. BMC Syst Biol 2008;2:87.
[3] Baßler J, Hurt E. Eukaryotic ribosome assembly. Annu Rev Biochem 2019;88(1):281–306.
[4] Gonzalez A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. EMBO J 2017;36(4):397–408.
[5] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;169(2):361–71.
[6] Laribee RN. Transcriptional and epigenetic regulation by the mechanistic target of rapamycin complex 1 pathway. J Mol Biol 2018;430(24):4874–90.
[7] Tafur L, Kefauver J, Loewith R. Structural Insights into TOR Signaling. Genes (Basel) 2020;11(8):885. https://doi.org/10.3390/genes11080885.
[8] Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 1991;253(5022):905–9.
[9] Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, et al. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 2002;10(3):457–68.
[10] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168(6):960–76.
[11] Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 1994;78(1):35–43.
[12] Schmidt A, Kunz J, Hall MN. TOR2 is required for organization of the actin cytoskeleton in yeast. Proc Natl Acad Sci USA 1996;93(24):13780–5.
[13] Betz C, Hall MN. Where is mTOR and what is it doing there? J Cell Biol 2013;203(4):563–74.
[14] Binda M, Péli-Gulli M-P, Bonfils G, Panchaud N, Urban J, Sturgill TW, et al. The Vam6 GEF controls TORC1 by activating the EGO complex. Mol Cell 2009;35(5):563–73.
[15] Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, Deloche O, et al. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. Mol Cell 2007;26(5):663–74.
[16] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008;320(5882):1496–501.
[17] Loewith R, Hall MN. Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 2011;189(4):1177–201.
[18] De Virgilio C, Loewith R. The TOR signalling network from yeast to man. Int J Biochem Cell Biol 2006;38(9):1476–81.
[19] Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell 2010;40(2):310–22.
[20] Ghosh S, Tergaonkar V, Rothlin CV, Correa RG, Bottero V, Bist P, et al. Essential role of tuberous sclerosis genes TSC1 and TSC2 in NF-κB activation and cell survival. Cancer Cell 2006;10(3):215–26.
[21] Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell 2010;40(2):179–204.
[22] Sarbassov DD, Ali SM, Sengupta S, Sheen J-H, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22(2):159–68.
[23] Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 2012;335(6076):1638–43.
[24] Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 1996;273(5272):239–42.
[25] Kasahara K, Nakayama R, Shiwa Y, Kanesaki Y, Ishige T, Yoshikawa H, et al. Fpr1, a primary target of rapamycin, functions as a transcription factor for ribosomal protein genes cooperatively with Hmo1 in *Saccharomyces cerevisiae*. PLoS Genet 2020;16(6):e1008865.
[26] Wei Y, Tsang CK, Zheng XFS. Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1. EMBO J 2009;28(15):2220–30.
[27] Tsang CK, Liu H, Zheng XFS. mTOR binds to the promoters of RNA polymerase I-and III-transcribed genes. Cell Cycle 2010;9(5):953–7.
[28] Vannini A, Ringel R, Kusser AG, Berninghausen O, Kassavetis GA, Cramer P. Molecular basis of RNA polymerase III transcription repression by Maf1. Cell 2010;143(1):59–70.
[29] Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev 2004;18(4):423–34.
[30] Claypool JA, French SL, Johzuka K, Eliason K, Vu L, Dodd JA, et al. Tor pathway regulates Rrn3p-dependent recruitment of yeast RNA polymerase I to the promoter but does not participate in alteration of the number of active genes. Mol Biol Cell 2004;15(2):946–56.

[31] Li H, Tsang CK, Watkins M, Bertram PG, Zheng XFS. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. Nature 2006;442 (7106):1058–61.
[32] Jorgensen P, Rupeš I, Sharom JR, Schneper L, Broach JR, Tyers M. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev 2004;18(20):2491–505.
[33] Huber A, French SL, Tekotte H, Yerlikaya S, Stahl M, Perepelkina MP, et al. Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L. EMBO J 2011;30(15):3052–64.
[34] Bosio MC, Fermi B, Spagnoli G, Levati E, Rubbi L, Ferrari R, et al. Abf1 and other general regulatory factors control ribosome biogenesis gene expression in budding yeast. Nucleic Acids Res. 2017;45(8):4493-506.
[35] Knight B, Kubik S, Ghosh B, Bruzzone MJ, Geertz M, Martin V, et al. Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. Genes Dev 2014;28(15):1695–709.
[36] Grove A. Control of RNA polymerase II-transcribed genes by direct binding of TOR kinase. Curr Genet 2018;64(1):131–5.
[37] Zencir S, Dilg D, Rueda MP, Shore D, Albert B. Mechanisms coordinating ribosomal protein gene transcription in response to stress. Nucleic Acids Res 2020;48(20):11408–20.
[38] Chauvin C, Koka V, Nouschi A, Mieulet V, Hoareau-Aveilla C, Dreazen A, et al. Ribosomal protein S6 kinase activity controls the ribosome biogenesis transcriptional program. Oncogene 2014;33(4):474–83.
[39] Recasens-Alvarez C, Alexandre C, Kirkpatrick J, Nojima H, Huels DJ, Snijders AP, et al. Ribosomopathy-associated mutations cause proteotoxic stress that is alleviated by TOR inhibition. Nat Cell Biol 2021;23(2):127–35.
[40] Bryant CJ, Lorea CF, de Almeida HL, Jr., Weinert L, Vedolin L, Pinto EVF, et al. Biallelic splicing variants in the nucleolar 60S assembly factor RBM28 cause the ribosomopathy ANE syndrome. Proc Natl Acad Sci U S A. 2021;118(19).
[41] Kampen KR, Sulima SO, Vereecke S, De Keersmaecker K. Hallmarks of ribosomopathies. Nucleic Acids Res 2020;48(3):1013–10128.
[42] Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Biosci 2020;10:31.
[43] Nakao A, Yoshihama M, Kenmochi N. RPG: the ribosomal protein gene database. Nucleic Acids Res. 2004;32(suppl_1):D168-D70.
[44] Mullis A, Lu Z, Zhan Y, Wang T-Y, Rodriguez J, Rajeh A, et al. Parallel concerted evolution of ribosomal protein genes in Fungi and its adaptive significance. Mol Biol Evol. 2020;37(2):455-68.
[45] Thompson MK, Rojas-Duran MF, Gangaramani P, Gilbert WV. The ribosomal protein Asc1/RACK1 is required for efficient translation of short mRNAs. Elife 2016;5. https://doi.org/10.7554/eLife.11154.
[46] Nilsson J, Sengupta J, Frank J, Nissen P. Regulation of eukaryotic translation by the RACK1 protein: a platform for signalling molecules on the ribosome. EMBO Rep 2004;5(12):1137–41.
[47] Wapinski I, Pfiffner J, French C, Socha A, Thompson DA, Regev A. Gene duplication and the evolution of ribosomal protein gene regulation in yeast. Proc Natl Acad Sci U S A 2010;107(12):5505–10.
[48] Dharia AP, Obla A, Gajdosik MD, Simon A, Nelson CE, Robinson-Rechavi M. Tempo and mode of gene duplication in mammalian ribosomal protein evolution. PLoS ONE 2014;9(11):e111721.
[49] Kellis M, Birren BW, Lander ES. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. Nature 2004;428 (6983):617–24.
[50] Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, et al. Genome evolution in yeasts. Nature 2004;430(6995):35–44.
[51] Dietrich FS, Voegeli S, Brachat S, Lerch A, Gates K, Steiner S, et al. The Ashbya gossypii genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. Science 2004;304(5668):304–7.
[52] Dean EJ, Davis JC, Davis RW, Petrov DA, McVean G. Pervasive and persistent redundancy among duplicated genes in yeast. PLoS Genet 2008;4(7): e1000113.
[53] Evangelisti AM, Conant GC. Nonrandom survival of gene conversions among yeast ribosomal proteins duplicated through genome doubling. Genome Biol Evol 2010;2(0):826–34.
[54] Shi Z, Fujii K, Kovary KM, Genuth NR, Röst HL, Teruel MN, et al. Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. Mol Cell 2017;67(1):71–83.e7.
[55] Ghulam MM, Catala M, Abou ES. Differential expression of duplicated ribosomal protein genes modifies ribosome composition in response to stress. Nucleic Acids Res 2020;48(4):1954–68.
[56] Fu Y, Deiorio-Haggar K, Anthony J, Meyer MM. Most RNAs regulating ribosomal protein biosynthesis in *Escherichia coli* are narrowly distributed to Gammaproteobacteria. Nucleic Acids Res 2013;41(6):3491–503.
[57] Shore D, Zencir S, Albert B. Transcriptional control of ribosome biogenesis in yeast: links to growth and stress signals. Biochem Soc Trans 2021;49 (4):1589–99.
[58] Parenteau J, Maignon L, Berthoumieux M, Catala M, Gagnon V, Abou Elela S. Introns are mediators of cell response to starvation. Nature 2019;565 (7741):612–7.
[59] Martin DE, Soulard A, Hall MN. TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell 2004;119 (7):969–79.
[60] Wade JT, Hall DB, Struhl K. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 2004;432(7020):1054–8.
[61] Kamau E, Bauerle KT, Grove A. The *Saccharomyces cerevisiae* high mobility group box protein HMO1 contains two functional DNA binding domains. J Biol Chem 2004;279(53):55234–40.
[62] Hall DB, Wade JT, Struhl K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in *Saccharomyces cerevisiae*. Mol Cell Biol 2006;26(9):3672–9.
[63] Reja R, Vinayachandran V, Ghosh S, Pugh BF. Molecular mechanisms of ribosomal protein gene coregulation. Genes Dev 2015;29(18):1942–54.
[64] Fingerman I, Nagaraj V, Norris D, Vershon AK. Sfp1 plays a key role in yeast ribosome biogenesis. Eukaryot Cell 2003;2(5):1061–8.
[65] Mallick J, Whiteway M. The evolutionary rewiring of the ribosomal protein transcription pathway modifies the interaction of transcription factor heteromer Ifh1-Fhl1 (interacts with forkhead 1-forkhead-like 1) with the DNA-binding specificity element. J Biol Chem 2013;288(24):17508–19.
[66] Albert B, Tomassetti S, Gloor Y, Dilg D, Mattarocci S, Kubik S, et al. Sfp1 regulates transcriptional networks driving cell growth and division through multiple promoter-binding modes. Genes Dev 2019;33(5-6):288–93.
[67] Zhu C, Byers KJRP, McCord RP, Shi Z, Berger MF, Newburger DE, et al. High-resolution DNA-binding specificity analysis of yeast transcription factors. Genome Res 2009;19(4):556–66.
[68] Xiao L, Grove A. Coordination of Ribosomal Protein and Ribosomal RNA Gene Expression in Response to TOR Signaling. Curr Genom 2009;10(3):198–205.
[69] Kasahara K, Ohtsuki K, Ki S, Aoyama K, Takahashi H, Kobayashi T, et al. Assembly of regulatory factors on rRNA and ribosomal protein genes in *Saccharomyces cerevisiae*. Mol Cell Biol 2007;27(19):6686–705.
[70] Berger AB, Decourty L, Badis Gwenaël, Nehrbass U, Jacquier A, Gadal O. Hmo1 is required for TOR-dependent regulation of ribosomal protein gene transcription. Mol Cell Biol 2007;27(22):8015–26.
[71] Xiao L, Kamau E, Donze D, Grove A. Expression of yeast high mobility group protein HMO1 is regulated by TOR signaling. Gene 2011;489(1):55–62.
[72] Panday A, Xiao L, Grove A. Yeast high mobility group protein HMO1 stabilizes chromatin and is evicted during repair of DNA double strand breaks. Nucleic Acids Res 2015;43(12):5759–70.
[73] Panday A, Grove A. Yeast HMO1: Linker Histone Reinvented. Microbiol Mol Biol Rev 2017;81(1). https://doi.org/10.1128/MMBR.00037-16.
[74] Gadal O, Labarre S, Boschiero C, Thuriaux P. Hmo1, an HMG-box protein, belongs to the yeast ribosomal DNA transcription system. EMBO J. 2002;21 (20):5498-507.
[75] Merz K, Hondele M, Goetze H, Gmelch K, Stoeckl U, Griesenbeck J. Actively transcribed rRNA genes in *S. cerevisiae* are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. Genes Dev 2008;22(9):1190–204.
[76] Rudra D, Zhao Yu, Warner JR. Central role of Ifh1p–Fhl1p interaction in the synthesis of yeast ribosomal proteins. EMBO J 2005;24(3):533–42.
[77] Cherel I, Thuriaux P. The IFH1 gene product interacts with a fork head protein in *Saccharomyces cerevisiae*. Yeast 1995;11(3):261–70.
[78] Dolinski KJ, Heitman J. Hmo1p, a high mobility group 1/2 homolog, genetically and physically interacts with the yeast FKBP12 prolyl isomerase. Genetics 1999;151(3):935–44.
[79] Kasahara K, Ki S, Aoyama K, Takahashi H, Kokubo T. *Saccharomyces cerevisiae* HMO1 interacts with TFIID and participates in start site selection by RNA polymerase II. Nucleic Acids Res 2008;36(4):1343–57.
[80] Kasahara K, Ohyama Y, Kokubo T. Hmo1 directs pre-initiation complex assembly to an appropriate site on its target gene promoters by masking a nucleosome-free region. Nucleic Acids Res 2011;39(10):4136–50.
[81] Bender A, Pringle JR. Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol Cell Biol 1991;11(3):1295–305.
[82] Engel SR, Dietrich FS, Fisk DG, Binkley G, Balakrishnan R, Costanzo MC, et al. The reference genome sequence of *Saccharomyces cerevisiae*: then and now. G3 (Bethesda). 2014;4(3):389-98.
[83] Louis EJ. Historical Evolution of Laboratory Strains of *Saccharomyces cerevisiae*. Cold Spring Harb Protoc. 2016;2016(7).
[84] Schawalder SB, Kabani M, Howald I, Choudhury U, Werner M, Shore D. Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature 2004;432(7020):1058–61.
[85] Albert B, Kos-Braun IC, Henras AK, Dez C, Rueda MP, Zhang Xu, et al. A ribosome assembly stress response regulates transcription to maintain proteome homeostasis. Elife 2019;8:e45002. https://doi.org/10.7554/eLife.45002.
[86] Tye BW, Churchman LS, Weis K. Hsf1 activation by proteotoxic stress requires concurrent protein synthesis. Mol Biol Cell 2021;32(19):1800–6.
[87] Panday A, Gupta A, Srinivasa K, Xiao L, Smith MD, Grove A, et al. DNA damage regulates direct association of TOR kinase with the RNA polymerase II-transcribed HMO1 gene. Mol Biol Cell 2017;28(18):2449–59.
[88] Haruki H, Nishikawa J, Laemmli UK. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. Mol Cell 2008;31 (6):925–32.
[89] Hermann-Le Denmat S, Werner M, Sentenac A, Thuriaux P. Suppression of yeast RNA polymerase III mutations by FHL1, a gene coding for a fork head protein involved in rRNA processing. Mol Cell Biol 1994;14(5):2905–13.
[90] Albert B, Knight B, Merwin J, Martin V, Ottoz D, Gloor Y, et al. A molecular titration system coordinates ribosomal protein gene transcription with ribosomal RNA synthesis. Mol Cell 2016;64(4):720–33.

[91] Kim MS, Hahn J-S. Role of CK2-dependent phosphorylation of Ifh1 and Crf1 in transcriptional regulation of ribosomal protein genes in *Saccharomyces cerevisiae*. Biochim Biophys Acta 2016;1859(8):1004–13.
[92] Kornprobst M, Turk M, Kellner N, Cheng J, Flemming D, Koš-Braun I, et al. Architecture of the 90S Pre-ribosome: A Structural View on the Birth of the Eukaryotic Ribosome. Cell 2016;166(2):380–93.
[93] Rudra D, Mallick J, Zhao Yu, Warner JR. Potential interface between ribosomal protein production and pre-rRNA processing. Mol Cell Biol 2007;27 (13):4815–24.
[94] Downey M, Knight B, Vashisht A, Seller C, Wohlschlegel J, Shore D, et al. Gcn5 and sirtuins regulate acetylation of the ribosomal protein transcription factor Ifh1. Curr Biol 2013;23(17):1638–48.
[95] Cai L, McCormick M, Kennedy B, Tu B. Integration of multiple nutrient cues and regulation of lifespan by ribosomal transcription factor Ifh1. Cell Rep 2013;4(6):1063–71.
[96] Cliften PF, Fulton RS, Wilson RK, Johnston M. After the duplication: gene loss and adaptation in *Saccharomyces genomes*. Genetics 2006;172(2):863–72.
[97] Lynch M, Conery JS. The evolutionary fate and consequences of duplicate genes. Science 2000;290(5494):1151–5.
[98] Conant GC, Wolfe KH. Turning a hobby into a job: How duplicated genes find new functions. Nature Rev Genet 2008;9(12):938–50.
[99] Hershberg R, Margalit H. Co-evolution of transcription factors and their targets depends on mode of regulation. Genome Biol 2006;7(7):R62.
[100] Hose J, Yong CM, Sardi M, Wang Z, Newton MA, Gasch AP. Dosage compensation can buffer copy-number variation in wild yeast. eLife. 2015;4:e05462.
[101] Lan X, Pritchard JK. Coregulation of tandem duplicate genes slows evolution of subfunctionalization in mammals. Science 2016;352(6288):1009–13.
[102] Zeevi D, Sharon E, Lotan-Pompan M, Lubling Y, Shipony Z, Raveh-Sadka T, et al. Compensation for differences in gene copy number among yeast ribosomal proteins is encoded within their promoters. Genome Res 2011;21 (12):2114–28.
[103] Liu J, Perumal NB, Oldfield CJ, Su EW, Uversky VN, Dunker AK. Intrinsic disorder in transcription factors. Biochemistry 2006;45(22):6873–88.
[104] Bose T, Lee KK, Lu S, Xu B, Harris B, Slaughter B, et al. Cohesin proteins promote ribosomal RNA production and protein translation in yeast and human cells. PLoS Genet 2012;8(6):e1002749.
[105] Kumar S, Mashkoor M, Balamurugan P, Grove A. Intricate regulation of ribosome biogenesis genes in response to mTORC1 signaling. bioRxiv 2021. https://doi.org/10.1101/2021.01.04.425249.
[106] Zhao Yu, McIntosh KB, Rudra D, Schawalder S, Shore D, Warner JR. Fine-structure analysis of ribosomal protein gene transcription. Mol Cell Biol 2006;26(13):4853–62.
[107] Chapal M, Mintzer S, Brodsky S, Carmi M, Barkai N, Hurst LD. Resolving noise-control conflict by gene duplication. PLoS Biol 2019;17(11):e3000289.
[108] Cherry JM, Adler C, Ball C, Chervitz SA, Dwight SS, Hester ET, et al. SGD: saccharomyces genome database. Nucleic Acids Res 1998;26(1):73–9.
[109] Teem JL, Abovich N, Kaufer NF, Schwindinger WF, Warner JR, Levy A, et al. A comparison of yeast ribosomal protein gene DNA sequences. Nucleic Acids Res 1984;12(22):8295–312.
[110] Leer RJ, Van Raamsdonk-Duin MMC, Mager WH, Planta RJ. Conserved sequences upstream of yeast ribosomal protein genes. Current Genet 1985;9(4):273–7.
[111] Lustig AJ, Kurtz S, Shore D. Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. Science 1990;250 (4980):549–53.
[112] Lascaris R, Mager W, Planta R. DNA-binding requirements of the yeast protein Rap1p as selected in silico from ribosomal protein gene promoter sequences. Bioinformatics 1999;15(4):267–77.