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Characterisation of the biological response of *Saccharomyces cerevisiae* to the loss of an allele of the eukaryotic initiation factor 4A

Veronica Venturi [1], Richard Little, Peter W. Bircham [2], Juliana Rodigheri Brito, Paul H. Atkinson, David R. Maass, Paul H. Teesdale-Spittle*

Centre for Biodiscovery, Victoria University of Wellington, Wellington, New Zealand

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ABSTRACT

The translation initiation machinery is emerging as an important target for therapeutic intervention, with potential in the treatment of cancer, viral infections, and muscle wasting. Amongst the targets for pharmacological control of translation initiation is the eukaryotic initiation factor 4A (eIF4A), an RNA helicase that is essential for cap-dependent translation initiation. We set out to explore the system-wide impact of a reduction of functional eIF4A. To this end, we investigated the effect of deletion of *TIF1*, one of the duplicate genes that produce eIF4A in yeast, through synthetic genetic array interactions and system-wide changes in GFP-tagged protein abundances. We show that there is a biological response to deletion of the *TIF1* gene that extends through the proteostasis network. Effects of the deletion are apparent in processes as distributed as chromatin remodelling, ribosome biogenesis, amino acid metabolism, and protein trafficking. The results from this study identify protein complexes and pathways that will make ideal targets for combination therapies with eIF4A inhibitors.

1. Introduction

The translation initiation machinery is emerging as a relevant, common target for therapeutic interventions towards seemingly unrelated diseases. Promising strategies are directed at the initiation complex by hindering the function of factors involved in canonical translation. The most abundant of these is the eukaryotic initiation factor 4A (eIF4A) [1], which exhibits helicase and cap-associating activities and its inhibition has been actively explored as a route to treating cancer [2], viral infections [3], and cachexia [4]. The eIF4A protein allows resolution of secondary structure in the 5′ untranslated region (UTR) of transcripts so that assembly of the preinitiation complex can occur and cap-dependent ribosomal translation can initiate [5]. Since the discovery of the marine metabolite pateamine A as an inhibitor of eIF4A [6] there has been growing interest in eIF4A as a drug target. Much of this research has been focussed on the potential to treat cancer through complete inhibition of the function of eIF4A, leading to loss of cap-dependent translation [2]. More recently, it has been shown that eIF4A inhibitors have transcript-dependent effects on translation, leading to the selective failure to produce proteins from transcripts with highly structured 5′ UTRs [7]. This has raised the potential of pharmacological regulation of eIF4A in the treatment of not only cancer [2] but also in the treatment of cachexia through fine-tuning of protein production at inhibitor concentrations well below levels where toxicity occurs [4].

In *Saccharomyces cerevisiae*, the two genes responsible for eIF4A production, *TIF1* and *TIF2*, encode an identical protein [8]. In order to understand the system-wide consequences of modification in abundance of functional eIF4A, we have dissected out the biological impact of removing the *TIF1* paralogue and here below we report the biological consequences of this genetic ablation. These are revealed through application of a Synthetic Genetic Array (SGA) analysis approach [9] and analysis of changes in system-wide protein abundance [10]. In particular, we propose that adaptation to the deletion of *TIF1* creates ripples through the translational network that extends beyond translation initiation and includes regulation of transcription and protein trafficking. The results from this study identify protein complexes and pathways that are potential targets for combination therapies with eIF4A inhibitors.

* Corresponding author.
E-mail address: paul.teesdale-spittle@vuw.ac.nz (P.H. Teesdale-Spittle).
[1] Current address: Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona 80003, Spain.
[2] Current address: Zentrum für Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

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2. Materials and methods

Strains of *S. cerevisiae* and plasmids used for SGA analysis were kindly gifted from Charles Boone (University of Toronto, Canada) as part of the YKO *MAT***a** or YKO *MATα* strain collection [11].

2.1. Mass spectrometry

BY4741 control and *tif1Δ* strains were grown in YPD media to log-phase under standard protocols [12]. Cells were lysed and proteins precipitated using chloroform methanol. The abundance of eIF4A in two technical replicates for each of three biological replicate samples was determined by mass spectrometry [13] using a Dionex UltiMateTM 3000 nano liquid chromatography (Nano LC) system feeding into a Linear Trap Quadrupole Orbitrap XL mass spectrometer via a nanospray ion source (Thermo Fisher Scientific, USA) followed by TIC for all identified eIF4A peptides [14] in Scaffold Q+ (4.4.8).

2.2. Synthetic genetic array, image analysis and data processing

The construction of *tif1Δ::natR* SGA query strain was accomplished via the non-essential gene switching method [14]. SGA analysis was performed according to an established protocol [15]. Images of 1536-colony plates were analysed by sequential steps including gridding, segmenting, and digitizing. Colony sizes were recorded using HT Colony software [16] and then uploaded into a MySQL database. The fitness of each double deletion mutant strain was determined by analysis of the images of the final *MAT***a** double mutant SGA plates. An in-house statistical algorithm, SESA, was used to score interactions [17]. Synthetic sick and lethal interactions as well as positive genetic interactions were identified by assigning a degree of reliability based on comparison to a set of *ura3Δ:natR* control SGAs. Genes were excluded from consideration if they met any of the following criteria: The deletion strain shows growth defects when crossed with the wild-type background strain; the gene is annotated as a dubious ORF, unless it overlaps partially or totally with an ORF of annotated function; the deletion strain was found to cause synthetic lethality or sickness in more than three SGA experiments based on unrelated query strains performed in our laboratory; or the gene was part of the linkage groups surrounding the deletion of *TIF1* or *TIF2* genes. Where possible, validation of putative genetic interactions was performed via tetrad dissection analysis following standard methods [18].

2.3. Production of a tif1Δ GFP library

A dual nuclear/cytoplasm RFP marker system was integrated into a commercially available GFP-fusion protein library (Invitrogen) by mating against the strain yCG253, carrying a nuclear localisation signal (NLS) linked with RedStar2 and a cytoplasmic mCherry [17], using a modified SGA methodology as described previously [19]. Secondly, a *tif1*Δ deletion was additionally integrated into the modified GFP collection in a second SGA procedure by mating with the *tif1*Δ::KanR strain from the OpenBiosystems *MATα* yeast knock out collection. Each resultant *MAT***a** *tif1*Δ haploid progeny carried an mCherry cytoplasmic marker, a RedStar 2 nuclear marker and one of the GFP-fusion proteins from the GFP library (*MAT***a**; XXX-GFP_*HIS3*, *tif1*Δ:*kanR*, *can1*Δ::STE2pr-Sp_URA; *lyp1*Δ::mCherry-Nat; *his3*Δ1; *leu2*Δ0; *ura3*Δ0::NLS-RedStar2-HPH; LYS2+).

2.4. Yeast GFP screen image acquisition and analysis

Synthetic media deficient in histidine containing 2% glucose, synthetic complete media with 2% glucose (SC), and agar plates were prepared following standard methods [20]. Imaging of GFP-fusion protein libraries were carried out as previously described [17] with some alterations. Libraries were pinned onto SD-histidine plates and grown for 16 h at 30 °C. Colonies were transferred to 384 well clear bottom plates (Perkin Elmer Cell Carrier) and grown in liquid SC medium for a further 4 h at 30 °C. Images were acquired using a Perkin Elmer Opera microscope using a 60X water immersion lens. GFP and RFP were excited using 488 nm and 561 nm lasers with emission fluorescence collected through 520/35 and 600/40 bandpass filters respectively. Automated segmentation was performed using the Acapella (Perkin Elmer) scripts developed previously [17], as outlined in Supplementary Table 2.

2.5. Deciphering functionally grouped genes

Ontology enrichment of genes leading to synthetic sickness or lethality with deletion of the *TIF1* genes or decreasing in abundance in a *tif1*Δ background were analysed by Yeastmine [21], compared to a background comprising the gene list of the parent SGA or GFP libraries as appropriate. Associated statistical values were calculated according to the hypergeometric test and subjected to Holm-Bonferroni correction [22]. For the SGA analysis, genes that failed verification were excluded from analysis.

3. Results and discussion

3.1. Deletion of TIF1 results in a reduction in eIF4A protein abundance

The relative abundance of eIF4A in parental *S. cerevisiae* (BY4741) and the *tif1Δ* strain was determined by mass spectrometry. As expected, the reduction in gene dosage in the deletion strain led to a reduction in eIF4A protein abundance, with the deletion strain having a fold-change in eIF4A abundance of 0.54 compared to the parental strain ($p = 0.0053$, *t*-test). The *TIF1* deletion strain is therefore a valid model for evaluation of the impact of reduction in the concentration of functional eIF4A.

3.2. Synthetic lethal interactions occur between TIF1 and genes associated with protein production and localisation

The SGA approach enables high throughput analysis of the effects of gene deletions on growth phenotype when combined with a targeted deletion [9]. Analysis of the ontologies associated genes deletions that lead to positive and negative genetic interactions when combined with deletion of the *TIF1* gene reveal processes that are sensitive to a change in eIF4A abundance. Initial assessment of the SGA output revealed 112 hits (104 negative genetic interactions and 8 positive genetic interactions, supporting information Table S1). Following removal of genes that failed the exclusion criteria (see materials and methods) or that failed tetrad analysis validation, these reduced to 28 high confidence negative genetic interactions with the *TIF1* gene. A number of strains could not be confirmed by tetrad analysis due to low sporulation efficiency, and were retained. The UBI4 strain was also included in further consideration due to a literature record of its interaction with the eIF4A protein [23]. The full list of remaining genes interacting with *TIF1* is provided as Table 1. Where strains showing genetic interactions arise from deletion of a dubious open reading frame (ORF) that overlaps with a known ORF these were also retained, although subsequent ontology analysis was undertaken with the known ORF.

Deletion of *TIF1* has minimal effect on growth rate. It was therefore expected that the set of synthetic sick or lethal