Excised linear introns regulate growth in yeast

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Spliceosomal introns are ubiquitous non-coding RNAs that are typically destined for rapid debranching and degradation. Here we describe 34 excised introns in Saccharomyces cerevisiae that—despite being rapidly degraded in log-phase growth—accumulate as linear RNAs under either saturated-growth conditions or other stresses that cause prolonged inhibition of TORC1, which is a key integrator of growth signalling. Introns that become stabilized remain associated with components of the spliceosome and differ from other spliceosomal introns in having a short distance between their lariat branch point and 3′ splice site, which is necessary and sufficient for their stabilization. Deletion of these unusual introns is disadvantageous in saturated conditions and causes aberrantly high growth rates in yeast that are chronically challenged with the TORC1 inhibitor rapamycin. The reintroduction of native or engineered stable introns suppresses this aberrant rapamycin response. Thus, excised introns function within the TOR growth-signalling network of S. cerevisiae and, more generally, excised spliceosomal introns can have biological functions.

Spliceosomal introns are a defining feature of eukaryotic life; they are present in all known eukaryotic genomes and absent from all known non-eukaryotic genomes.1 Spliceosomal introns and splicing can have important roles in eukaryotic gene evolution, mRNA expression and protein diversification. Moreover, once excised from the primary transcript, introns can sometimes be further processed to generate non-coding RNAs, such as microRNAs or small nucleolar RNAs. In principle, full-length excised introns could also be a fertile source of functional non-coding RNAs. However, excised intron lariats are debranched and degraded within seconds, and thus are generally viewed as inert and ephemeral by-products of gene expression.

Functions for individual introns have been examined most thoroughly in the intron-poor budding yeast S. cerevisiae, which contains approximately 300 spliceosomal introns, with only a few annotated cases of alternative-splicing events. For most introns tested, no growth phenotypes are detected upon their removal, but for a few, phenotypes are observed. Some functions inferred from these phenotypes, such as an influence on gene expression, manifest entirely during pre-mRNA production and processing, and thus before the introns exist as separate RNA molecules. Other functions, such as providing a source of small nucleolar RNAs, occur after splicing, although in these cases the flanking portions of the intron are still rapidly catabolized. Thus, functional analyses in S. cerevisiae support the prevailing view that the collective fate of introns after splicing is solely to be debranched and at least partially degraded.

Although most yeast introns have now been individually assayed for function, relatively few experimental conditions have been explored. Most experiments assay cells during log-phase growth, which provides consistent measurements in a standardized system that is sensitive to detect differences in growth and metabolism. However, outside of the laboratory, yeast cells are unlikely to spend many consecutive generations rapidly dividing and more often face limiting nutrients or other stresses. Accordingly, we set out to examine the gene regulation of S. cerevisiae outside of the context of log-phase growth.

Accumulation of excised, linear introns

We performed RNA sequencing (RNA-seq) on two S. cerevisiae samples: one taken from a culture in log-phase growth and the other from a saturated culture in which cell density was minimally increasing. For most intron-containing genes (exemplified by ACT1), very few intron-mapping RNA-seq reads were observed from either culture condition (Fig. 1a), as expected if introns were rapidly degraded after splicing. However, for a subset of genes (exemplified by ECM33), many intron-mapping reads were observed specifically from the saturated culture (Fig. 1b). Indeed, the higher density of reads for the ECM33 intron compared to that for its exons suggested that in this growth condition the intron accumulated to much greater levels (tenfold) than did its corresponding mature mRNA.

Read coverage dropped abruptly at the 5′ and 3′ boundaries of the ECM33 intron (Fig. 1b, Extended Data Fig. 1), which suggested accumulation of full-length intron. To examine whether our RNA-seq analysis was detecting the accumulation of excised introns of a defined size, we probed RNA blots for the inferred RNA species. Probes to the ECM33 intron detected a single major species migrating at the position expected for the full-length intron (Fig. 1c). Likewise, a probe for SAC6, another intron inferred by RNA-seq to accumulate in saturated cultures (Fig. 1d), detected an RNA of the size expected for the full-length excised intron (Fig. 1e). These introns that accumulate in saturated cultures are hereafter called ‘stable introns.’

A known mechanism by which excised introns can be protected from degradation is by evading debranching and persisting as either lariat RNAs or circular derivatives in which the lariat tail is missing. However, these nonlinear species have either branched RNA or a 2′-5′ phosphodiester linkage, which would impede reverse transcription and thereby cause RNA-seq reads to be depleted in the region of the branch point—a pattern that we did not observe in the RNA-seq profiles (Fig. 1b, Extended Data Fig. 1). To test further whether ECM33 intronic RNA accumulated as either a lariat RNA or its circular derivative, we collected RNA from yeast that lacked Dbr1 (the enzyme that is required to debranch intron lariats) and compared the ECM33 intronic RNA that accumulated in the dbr1Δ strain with RNA that...
accumulated in wild-type saturated cultures. As expected, in dbr1 Δ cultures the ECM33 intron was detected as two abundant species, which corresponded to the branched lariat and its circular derivative (Fig. 1f). Importantly, neither of these nonlinear species co-migrated with the linear intron identified in wild-type saturated cultures. These results confirmed that Dbr1 is necessary to form linear introns outside of log phase and showed that the previously known mode of decreased intron turnover cannot explain the accumulation of excised introns in a saturated culture.

Another mechanism that might protect stable introns from degradation in saturated cultures is incorporation into a ribonucleoprotein complex. Indeed, the ECM33 intron predominantly co-sedimented with complexes that were about the size of ribosomal subunits (35–50 Svedberg units, Extended Data Fig. 2a). To identify proteins that associate with the intron, we performed pull-down experiments from gradient fractions that contained the intron and used quantitative mass spectrometry to identify co-purifying proteins. For these experiments, we took advantage of the observation that tagged versions of the ECM33 intron excised from expression constructs retained the behaviour of the endogenous ECM33 intron (Extended Data Fig. 2b, c). Each of the top 10 proteins that consistently co-purified with MS2-tagged versions of the ECM33 intron (average enrichment, 4.9-fold) were spliceosomal proteins, the identities of which indicated that the excised and debranched ECM33 intron resided in a specific complex; when compared to known spliceosome complexes, this complex most closely resembled the intron–lariat spliceosome (ILS) complex (Extended Data Table 1).

Studies in log-phase extracts indicate that introns are debranched after spliceosome disassembly. If stable introns were also debranched outside the spliceosome, they would then need to re-associate with spliceosome components as linear RNAs. A simpler model is one of continued association of stable introns with ILS components during their debranching; in this case, the relationship between ILS disassembly and debranching presumably varies depending on growth condition and/or intron identity. In either scenario, our findings indicated that, in saturated cultures, accumulating introns are bound to and presumably protected by a complex that resembles the ILS.

### Defining features of stable introns

We performed a systematic search for all introns that undergo a switch in stability and accumulate as linear RNAs in saturated cultures. In this search, RNA-seq reads of each intron-containing gene were analysed for a preponderance of reads that mapped to introns—particularly those that mapped to edges of excised introns (consistent with a post-splicing intron) relative to those that mapped across splice sites and splice junctions (which represented signatures of intron retention and mature mRNA expression, respectively) (Extended Data Fig. 3a). Inspection of RNA-seq reads that mapped to 3′ edges of stable introns identified many that were extended by one or more untemplated adenosine residues (Extended Data Fig. 3b). Short 3′-terminal oligoadenylate tails are added by the TRAMP complex to mark nuclear RNAs for degradation. Our finding that many stable-intron molecules had these tails suggested that these molecules might have been targeted for exosomal decay, yet had somehow escaped this decay. Regardless of their function, these untemplated adenosine residues provided an additional criterion for annotation of stable introns, which helped us to confidently identify another 28 introns that accumulated in saturated cultures (Extended Data Fig. 4, Extended Data Table 2).

We searched among the 30 stable introns for common features, which cellular machinery might use to differentiate them from the remaining introns that are rapidly degraded in saturated cultures. Compared to other introns, stable introns had similar strengths of canonical splicing motifs (Extended Data Fig. 5a), similar length distributions (Extended Data Fig. 5b), no common predicted structures or enriched sequence motifs (Extended Data Fig. 5c, d), and no enriched functional ontologies of their host genes. Of all the features we examined, the only one that differed was the distance between the lariat branch point and 3′ splice site (3′SS), which tended to be shorter for stable introns (Fig. 2a).

To investigate a potential role of branch-point position in influencing intron stability, we introduced mutations that changed endogenous branch point–3′SS distances and examined the effects on intron accumulation. Lengthening the short branch point–3′SS distance of the normally stable ECM33 intron from 25 to 45 nucleotides abrogated the accumulation of the full-length excised intron, which indicated that a short branch point–3′SS distance is not sufficient for stability of this intron (Fig. 2b). Moreover, shortening the branch point–3′SS distance of the normally unstable ACT1 intron from 44 to 25 nucleotides conferred stability to this intron in saturated culture, which suggested that a short branch point–3′SS distance is not only necessary but also sufficient for stability (Fig. 2b).

The notion that a short branch point–3′SS distance is sufficient for stability suggested that introns that had short branch point–3′SS distances yet were not identified as stable introns (Fig. 2a) might not have been transcribed during the period at which stable introns are protected from degradation. To investigate this possibility, we used our expression construct to express introns that had not been identified as stable introns, choosing two introns with a short (20–25 nucleotide) and two with a long (37- and 44-nucleotide) branch point–3′SS distance. The two with short branch point–3′SS distances accumulated
manner did not cause stable-intron formation (Extended Data Fig. 6a). Moreover, stable introns accumulated to lower levels in the TORC1-strain, which contains six hyperactive alleles of the regulatory network31 (Extended Data Fig. 7a)—results that further validated the involvement of TORC1 signalling.

To extend this investigation to the transcriptome, we prepared RNA-seq libraries from yeast that were treated for 4 h with rapamycin. We also investigated stable-intron formation in a metabolically different saturated-growth scenario: a lawn of yeast grown over 3 days in an aerobic environment. When compared to saturated-liquid culture, both rapamycin-treated cells and cells from the lawn showed a similar accumulation of not only the ECM33 intron (Extended Data Fig. 7b) but also other stable introns (Extended Data Fig. 7c). In total, 34 introns (11% of total S. cerevisiae introns) were classified as stable introns in at least one of the three conditions (Extended Data Fig. 4, Extended Data Table 2).

We tested several genetic and environmental perturbations for their effects on stable-intron accumulation. Gain-of-function mutations in TAP42 or SCH9 (the two major effector branches of TORC1) were individually insufficient to override TORC1 repression and attenuate stable-intron formation (Extended Data Fig. 6b, c), and Sch9 activity was not required for stable-intron regulation (Extended Data Fig. 6d), which implicated involvement of another effector branch of TORC1. Although depletion of carbon, nitrogen and amino acids rapidly inhibit facets of TORC1 signalling29, these conditions did not induce premature stable-intron formation (Extended Data Fig. 6e). Likewise, direct perturbations of the general amino acid control pathway did not disrupt stable-intron regulation (Extended Data Fig. 6f, g). Thus, the role of TORC1 in stable-intron regulation appeared separable from its role in rapid response to nutrient deprivation.

As illustrated for the ECM33 intron (Fig. 3d), stable introns were not detected after treating with rapamycin for 1 h—a time period that is sufficient to phenocopy the TORC1 response to nutrient deprivation. This result was consistent with our observation that some conditions that are known to rapidly inhibit aspects of TORC1 signalling did not induce stable introns, and motivated an attempt to account for this delayed response to rapamycin. Few studies have explored prolonged TORC1 inhibition in yeast, and those that report on ≥2-h rapamycin treatments observe notably different TORC1-mediated responses compared to short-term rapamycin treatment or starvation32–35. Some of these studies33–35 show that prolonged TORC1 inhibition (≥2-h treatment with rapamycin or tunicamycin) phenocopies secretory stress more closely than nutrient stress. We found that the secretory stressors tunicamycin and dithiothreitol (DTT) induced stable-intron formation, that for both stressors this induction required extended treatment durations similar to that required for rapamycin, and that induction of stable introns by these canonical activators of the unfolded-protein response did not require Ire1, the essential sensor of the unfolded-protein response (Fig. 3e, f). These results indicated that TORC1-mediated stable-intron accumulation depends on connections between TORC1 and membrane-trafficking or secretory stress36,37, and not the more-often-considered connections between TORC1 and nutrient stress.

**Biological function of stable introns**

The discovery of stable introns and their link to the TORC1 growth-signalling pathway brought to the fore the question of their function. To build strains to test for loss-of-function phenotypes, we used a CRISPR–Cas9 system38,39 that facilitated precise removal of introns without affecting exonic sequences. We reasoned that stable introns might contribute collectively to function, and therefore generated a quintuple mutant that lacked stable introns of five genes (ECM33, UBC4, HNT1, SAC6 and RFA2). Because introns from these genes normally accumulated to high levels, this strain, which we call EUHSR, had less than half the stable intron molecules as compared to the wild-type strain (Fig. 4a, Extended Data Fig. 8a).

To detect the consequences of reducing stable-intron abundance, we co-cultured the EUHSR and wild-type strains in a competitive-growth
Fig. 3 | Stable-intron accumulation occurs after prolonged TORC1 inhibition. a, Distinct accumulation patterns of the ECM33 stable intron and its host mRNA. After seeding at OD600 nm = 0.2, growth was monitored (left), and aliquots were collected for RNA-blot analysis (right). The blot is as in Fig. 1c, except it used a gel designed to resolve longer RNAs. The asterisk marks the migration of the 5.8S rRNA transcript. b, Accumulation of the ECM33 stable intron in cells cultured with rapamycin. Cultures were allowed to grow to early log phase, collected at indicated times and analysed as in Fig. 1c. n = 6 biological replicates. c, Requirement of rapamycin-sensitive TORC1 for accumulation of the ECM33 intron after 1 h of rapamycin treatment. Accumulation of this intron after 4 h of rapamycin treatment is shown for comparison. Otherwise, this panel is as in Fig. 1a. d, Accumulation of stable introns in cells cultured with 100 nM rapamycin (Rap), 5 mM dithiothreitol (DTT) or 1 μg ml⁻¹ tunicamycin (Tm). Otherwise, this panel is as in b, except that the RNA blot was also probed for the SAC6 intron. n = 3 biological replicates. e, Accumulation of stable introns in cells cultured with DTT or tunicamycin despite deletion of IRE1. Otherwise, this panel is as in e. n = 2 biological replicates.

Mechanistic possibilities

The discovery of this node of the TOR regulatory network raises mechanistic questions, including that of how stable-intron accumulation might inhibit growth. Our results imply that the stoichiometry of stable introns relative to some cellular component underlies this function. One possibility is that stable introns sequester spliceosomes to reduce splicing activity. Supporting this idea, stable introns associate with spliceosomes in cells cultured with rapamycin (Fig. 3b–f), and stable introns inhibit growth of cells with decreased splicing activity. Elevated levels of stable introns negatively regulate—nearly completely rescuing its defective response to rapamycin (Fig. 4e). Analogous results were observed when using stabilized and normal versions of the ACT1 intron (referred to as ACT1-stable and ACT1-unstable, respectively) (Figs. 4f, 4g). The ability of a stable version of the ACT1 intron (which does not exist naturally in S. cerevisiae) to rescue the quintuple-mutant phenotype confirmed that this phenotype was a primary consequence of reduced stable-intron abundance.

Taken together, our results show that stable introns function within the TORC1-mediated stress response in S. cerevisiae (Fig. 4g). TORC1 activity prevents stable-intron formation, as shown by the accumulation of these introns when cells undergo prolonged TORC1 inhibition (Fig. 3b–f), and stable introns inhibit growth of cells with decreased TORC1 activity, as shown by the increased growth observed in TORC1-inhibited strains with fewer stable introns (Fig. 4c–f). Thus, this double-negative regulation—in which TORC1 inhibits stable introns, which in turn inhibit growth—forms a previously unknown node of the TOR regulatory network, which works in concert with other TORC1-dependent and TORC1-independent pathways to control growth in S. cerevisiae (Fig. 4g).
Fig. 4 | Stable introns influence yeast fitness and growth. a, Stable-intron genomic deletions and their estimated effect on total stable-intron molecules. Strains were named using single-letter abbreviations of genes with deleted introns (shown in colour key). b, Altered fitness of the EUHSR strain. Left, co-cultures of wild type and EUHSR were diluted every three days (dashed lines). The fraction of wild type in each co-culture, measured before each dilution and a day after each dilution, is plotted. Lines for each biological replicate are coloured to indicate periods of saturated growth (blue), returning to and exiting rapid growth (orange), and initial post-seeding (black). Right, mean fold change of wild type (WT) compared with overexpression of a non-stable intron (ACT1) (Extended Data Fig. 8c, d). Future characterization of the stable-intron complex will provide more-detailed information on the cellular machinery that is sequestered and might also provide insight into other mechanistic questions, such as how stable introns are protected from degradation and how this protection is biochemically coupled to TORC1 inhibition.

Discussion

The ease with which we were able to observe the accumulation of stable introns raises the question of why they were not detected earlier. The answer lies with two features of our analysis. First, we examined cells in saturated culture, whereas most analyses examine cells in log phase—a stage at which all excised introns are rapidly degraded. Second, we avoided mRNA poly(A) selection, whereas many analyses perform poly(A) selection before RNA-seq; this selection depletes excised introns, which lack poly(A) tails. In addition, we used an in-house RNA-seq protocol in which RNA was fragmented, and fragments of 27- to 40-nucleotides in size were isolated for sequencing; by contrast, most analyses use commercial RNA-seq kits that deplete RNAs that are shorter than a few hundred nucleotides in size—the size of excised yeast introns. In principle, studies that used splicing-isomir microarrays to assay intron retention during stress, including rapamycin and DTT treatment, might have detected stable introns \(^{40,43,44}\). However, those studies focus on measurements over the course of 40 min, with no measurements taken beyond 2 h of treatment, which might not have been enough time to detect stable introns under these conditions. Our findings on prolonged TORC1 inhibition strengthen the links between TORC1 signalling and secondary stress in yeast \(^{35,39}\), and show that short versus long durations of TORC1 inhibition can result in distinct effects on some downstream biological processes—including stable-intron regulation.

Although our ideas regarding the mechanism of stable-intron function require validation, one interesting aspect of this mechanism is that it operates regardless of stable-intron sequence or genomic origin. This observation provides an example in which the history of a non-coding RNA (that is, how the molecule is born) can be sufficient to impart a cellular function—indeed, independently of any primary-sequence considerations. Similar principles might apply to other non-coding RNAs, especially those that lack primary-sequence conservation.

To evaluate this idea, we revisited our RNA-seq data from rapamycin-treated yeast with the goal of determining the aggregate level of stable introns relative to spliceosome components. This analysis showed that stable-intron molecules reached 40% of the level of U5 nuclear RNA. Thus, stable-intron accumulation was within a regime in which depleting 55% of stable-intron molecules—as in our EUHSR strain—could substantially alter spliceosome availability. Furthermore, overexpressing a stable intron (ECM33) in the EUHSR strain grown in rapamycin resulted in more intron retention and less accumulation of mRNAs of ribosomal protein genes as compared with overexpression of a non-stable intron (ACT1) (Extended Data Fig. 8c, d). Future

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0828-1.

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**METHODS**

**Yeast strains and genetic manipulations.** *S. cerevisiae* strains used in this study are listed in Supplementary Table 1. With the exception of strains acquired for TOR1-1 (Fig. 3c) and TORC1* (Extended Data Fig. 7a) experiments, all strains were in the BY4741 background. A TAP42 heterozygous diploid knockout strain (Horizon Discovery) was transformed with plasmids encoding either wild-type (TAP42) or mutant (tap42-11) alleles before sporulation and tetrad dissection. Transformations were performed using standard methods. Intron deletions and endogenous branch-point manipulations were made using a CRISPR–Cas9 system adapted for use in *S. cerevisiae*30,31 and were confirmed by colony PCR and Sanger sequencing of relevant genomic loci. Clones with correct deletions were counter-selected for the Cas9 plasmid by plating onto 5-fluoroorotic acid (5-FOA) medium before their use in experiments. This process was iterated to generate strains with multiple deleted introns. Strains that underwent 5-FOA counter-selection were confirmed to not be petite by tagging with a marker integrated into the YPS2-11 locus.

**RNA-seq and radiolabeling.** RNA-seq was performed using standard methods. RNA-seq libraries were prepared as described48 with Trizol (Life Technologies). Total RNA was extracted from lysate powder using TRI Reagent (Sigma-Aldrich), digested to generate cDNA using the M-MLV RT (Invitrogen) with a first-strand primer and a reverse transcription reaction. The resulting cDNA was used as a template for PCR with barcodes and Illumina adaptors. PCR products were purified using AMPure XP magnetic beads (Agilent Technologies) to reduce primer dimers and sequenced on an Illumina HiSeq 2500 using 150-bp paired-end reads. Sequencing was performed by the Broad Institute (Cambridge, MA, USA) and CSHL (Cold Spring Harbor, NY, USA). Reads were trimmed of adaptor sequence using cutadapt (v.8.1.0.0).

**Genetic analysis.** The presence of introns in each strain was determined by comparing the log-linear portion of each growth curve. Growth curves were calculated with the fasta-get-markov program from the MEME Suite. In addition, a search for enrichment of position-specific motifs was performed using the BASS tool54. To identify stable introns as the predominant isoform for many introns in stable-intron-inducing conditions, the relative abundance of wild type and EUHSR was measured by PCR across the intron-deletion loci (primers listed in Supplementary Table 3).

**Growth curves.** Growth curves were calculated using Nudge Edge 2.096 well plate reader software. All experiments were performed in quadruplicate. For each experiment, three to four 48-well plates were generated with a series of batch cultures, which were performed in biological replicate. The experiment started with a 50-ml co-culture in a 250-ml baffled flask seeded with an equal volume of thawed lysate powder (500–800 μg). The lysates were placed on a rotator mixer at 4 °C for 5 min to allow for resuspension. Following brief vortexing, 1 ml of thawed lysate powder (500–800 μg) was added to each well in 48-well plates. The cultures were seeded to OD600 nm of 0.2 from overnight cultures (typically OD600nm ~6) for growth to log phase or saturation. Log-phase cultures were collected during early log phase, typically at OD600nm of 0.5, reached 4–5 h after seeding. Unless otherwise indicated, saturated cultures were collected after 18–20 h after seeding. In acute nutrient-depletion experiments, cultures were grown to OD600 nm ~1, filtered, washed twice with water and resuspended in appropriate medium (SC–glucose, SC–ammonium sulfate, SC–leucine or SC–uracil). All cultures were rapidly collected by vacuum filtration and flash-frozen in liquid nitrogen as described37. Frozen pellets were mechanically lysed using a Sample Prep 6870 Freezer/Mill (Spx Sample Prep, 10 cycles of 2 min on, 2 min off at setting 10). Lysate powder was aliquoted and stored at −80°C. pGPoptem and EUHSR strains were grown to log phase in SC–Trp–G418 and SC–Pep42–HIS3 plates respectively for 48–72 h with shaking at 30 °C. Between 1 min on and 1 min off. Every strain tested on each plate was run in triplicate. Single wells were seeded at OD600 nm ~0.2 from early-log-phase cultures, and plates were incubated at 30°C. Absorbance was read every 5 min with shaking on the ‘background’ setting, cycling between 1 min on and 1 min off. Every strain tested on each plate was run in technical triplicate. Single wells were censored if artefactual spikes in OD600 nm attributable to bubbles or condensation were observed. Strains were censored if more than one well was censored. Biological replicates for each strain included at least two independently derived transfectants. A replicate of parental BY4741 was included on every plate as a control strain, and SC medium was included on every plate as a control condition for every strain being assayed. The growth rate was calculated from the log-linear portion of each growth curve. Growth curves were analysed with SkanIt (v.3.2).

**RNA blots.** Total RNA was extracted from lysate powder using TRI Reagent (Ambion) according to the manufacturer's protocol. Ten micrograms of total RNA for each sample was resolved on a denaturing polyacrylamide gel and transferred onto a Hybond membrane (GE Healthcare) using a semi-dry transfer cell. Because ultraviolet crosslinking is biased against shorter RNAs, EDC (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide; Sigma-Aldrich) was used to chemically crosslink 5' phosphates to the membrane55. Blots were hybridized to radio-labelled DNA probes. Probe oligonucleotides were listed in Supplementary Table 1. For experiments in Fig. 3a, RNAs were instead resolved on a glyoxyl agarose gel and transferred overnight onto a Nitran SuPerCharge Turboblotter membrane (GE Healthcare). RNA blot data were analysed with ImageQuant TL (v.8.1.0.0).

**Sedimentation velocity.** Crude lysates were prepared by resuspending an aliquot of thawed lysate powder (500–800 μg of loosely packed powder) in 1 ml of lysate buffer (20 mM Tris–HCl, pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 2 mM DTT, 20 U/ml SUPERase In (Ambion), and Complete EDTA-free Protease Inhibitor Cocktail (Roche)). The lysates were placed on a rotor mixer at 4°C for 5 min to allow for resuspension. Following brief vortexing,
lysates were centrifuged at 1,300g for 10 min, and the supernatant loaded onto a 12.5-mL linear 10–30% (w/v) sucrose gradient (20 mM HEPES–KOH (pH 7.4), 5 mM MgCl₂, 100 mM KCl, 2 mM DTT, and 20 U/mL SUPERaseIn). Gradients were centrifuged in a pre-chilled SW-41 Ti rotor at 38,000 rpm for 4 h at 4°C. Gradients were fractionated using a Piston Gradient Fractionator (Bio-comp) in 1-mL fractions. For RNA analysis, total RNA was extracted from a portion of each fraction using TRI Reagent. RNA blots were performed as described for total RNA, except RNA loading was normalized by per cent of gradient fraction rather than by RNA concentration. For pull-downs, fractions were flash-frozen and stored at −80°C.

Ectopic intron expression, affinity purification and mass spectrometry. Intron-overexpression constructs were based on SC–Ura-selectable pRSI146 (CEN) or pRSI1426 (2u) vectors58 (Extended Data Fig. 3c, Supplementary Table 2). Intron constructs were inserted in 49 nucleotides downstream of the URA3 start codon by Gibson assembly. Proper splicing was confirmed by RNA blot and cell viability, as cells unable to produce Ura3p through intron removal could not grow on SC–Ura medium. Pull-down experiments were performed in the ECM33 Δinttron strain. Introns were purified using two MS2 hairpins (2 × MS2) inserted in various positions within the ECM33 intron (Supplementary Table 5). To minimize potential effects on splicing, the sequence within 60 nt of the 5′ splice site and 80 nt of the branch point was kept constant across all constructs. The 2 × MS2 hairpin sequence was based on CRISPR RNA scaffold designs54. 3 × Flag-tagged MS2 coat protein with a C-terminal nuclear-localization signal (FLAG–MCP) was co-expressed from the constructs that also expressed the MS2-tagged introns.

We were unable to purify intact introns from the supernatant of saturated cultures owing to increased endogenous RNase activity in saturated cultures54. To circumvent this, sucrose-gradient fractionation containing the intron of interest (typically fractions 6–9, determined by RNA blot) were pooled and used as the starting material for purification. Anti-Flag M2 Magnetic Beads (Sigma–Aldrich) (20 μL of packed-bead volume) were equilibrated and washed twice in 10 volumes of buffer 1 (100 mM KCl, 20 mM HEPES KOH (pH 7.9), 1% Triton X-100, 20 U/mL SUPERaseIn, and Complete EDTA-free Protease Inhibitor Cocktail). Four hundred microlitres of the pooled fractions was added to the beads and incubated on a rotator mixer for 30 min at 4°C. Remaining at 4°C, the beads were washed twice in 10 volumes of buffer 1 and twice in 10 volumes of buffer 2 (200 mM KCl, 20 mM HEPES KOH (pH 7.9), 1% Triton X-100, 20 U/mL SUPERaseIn, and Complete EDTA-free Protease Inhibitor Cocktail). Bound Flag–MCP was eluted from beads with 30 μL of 150 ng/μL 3 × Flag peptide (Sigma–Aldrich) on a rotator mixer for 30 min at 4°C. The eluents were precipitated with TCA, digested with trypsin and labelled with tandem mass tags to allow for quantitative comparisons between six total samples (three control and three test samples). Peptides were analysed by liquid chromatography–tandem mass spectrometry using an Orbitrap Elite (Thermo Fisher) coupled with a NanoAcquity UPLC system (Waters). Peptides were identified using SEQUEST and data analysed using PEAKS Studio (Bioinformatics Solutions).

Experimental design and reproducibility. No statistical methods were used to predetermine sample size. Growth-curve cultures were randomized by permutation of strain placement on 96-well plates across experiments. Edge effects were not significant within the time span that measurements were taken. The investigators were not blinded to allocation during experiments and outcome assessment. RNA-seq results for biological replicates correlated well (log-phase culture, R² = 0.98 (mRNA, n = 5,652); saturated culture, R² = 0.88 (mRNA, n = 5,762) and 0.78 (stable introns, n = 30)).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequencing data and the processed data for each gene and intron are available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE121765. For gel source data, see Supplementary Fig. 1. All other data are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | RNA-seq coverage across the branch point and to the 3′SS of the ECM33 intron. The pileup of reads mapping to the 61-nucleotide region centred on the ECM33 branch point, replotted from Fig. 1b, is shown (red, top). The position of the branch point (closed circle with flanking dashed lines) is indicated on the intron (thick grey line) and relative to the 3′ exon (grey box). Bottom, all reads mapping uniquely to this region are shown (thin grey lines). Reads mapping across the exon–exon junction are coloured blue in the region of the excised intron and are shown above the other reads. Mismatched nucleotides within reads are indicated with coloured bars, with the colour of the bar indicating the identity of the mismatch. Terminal untemplated nucleotides have been clipped from reads.
Extended Data Fig. 2 | Stable-intron sedimentation and expression constructs for pull-down and mass spectrometry. a, Co-sedimentation of the ECM33 stable intron with large ribonucleoprotein complexes. Cleared lysate from a saturated yeast culture was fractionated by sedimentation into a 10–30% sucrose gradient, and RNA was extracted from each fraction. An RNA blot that resolved 25% of the RNA from each fraction and was probed for the ECM33 intron is shown. Fractions are oriented with increasing sedimentation from left to right. RNA was also extracted from 12.5% of the total lysate before sedimentation, of which 50% was loaded for comparison (left lane). The sedimentation of 40S and 60S complexes is marked on the basis of the location of ribosome subunits. Migration of markers with lengths indicated (in nucleotides) is shown on the left. \( n = 6 \) biological replicates. b, Behaviour of stable introns ectopically expressed from the URA3 gene. An RNA blot probed for the ECM33 intron after resolving total RNA from cultures expressing the ECM33 intron from the endogenous ECM33 locus (WT, left lanes), cultures from a strain in which the intron had been deleted (ECM33Δintron, middle lanes) and cultures of this deletion strain ectopically expressing the ECM33 intron spliced from the plasmid-borne URA3 reporter gene (right lanes) is shown. pRS416-ECM33_intron plasmid shown on right; \( P_{URA3} \), URA3 promoter; \( CEN/ARS \) (centromere/autonomously replicating sequence), low-copy origin of replication. Interior lanes that were not relevant to this experiment were removed from this image (vertical line). Otherwise, this panel is as in Fig. 1c. \( n = 4 \) biological replicates. c, Behaviour of stable introns with MS2 hairpins inserted to be used as affinity tags for pull-downs. Five different plasmids with a common backbone (right; \( P_{TEF1} \), TEF1 promoter; FLAG–MCP, coding region of Flag-tagged MS2 coat protein) each expressed URA3 with a different variant of the ECM33 intron (variants A–E, schematized below). These plasmids were each expressed in the strain that lacked an endogenous ECM33 intron (ECM33Δintron). The RNA blot resolved total RNA from the indicated cultures and was probed for a sequence common to the intron variants; otherwise, as in Fig. 1c. The 2 × MS2 hairpin region is 90 nucleotides long, and the expected linear-intron sizes were: A, no intron; B, 330 nucleotides; C, 420 nucleotides; D, 300 nucleotides; and E, 420 nucleotides. \( n \geq 2 \) biological replicates.
Extended Data Fig. 3 | Stable introns have oligoadenylate tails. 

a, Example of RNA-seq support for intron accumulation and terminal adenylylation. The diagram (top, left) classifies the RNA-seq reads that derive from the possible intron states of ECM33 transcripts (black lines, reads; red dashed lines, intron boundaries). Read counts from log-phase and saturated cultures (normalized for library depth) are listed for each class of reads (top, right). For convenient comparison to intron accumulation, exon reads are only counted if they map within 165 nucleotides of either splice site (thereby encompassing 330 nucleotides, the length of the intron). The alignment (bottom) shows representative reads mapping to the intron 3′ terminus, aligned below the sequence of the 3′ intron–exon junction. Many of these reads had untemplated terminal adenosine residues (blue). b, Composition of untemplated tailing nucleotides observed in a saturated culture. Reads that had at least one terminal untemplated nucleotide after trimming the 3′ adaptor sequence were collected, and the position of this tail was annotated as that of the last templated nucleotide. Counts of reads with tails added at positions 0 to +20 relative to the 5′ss, −10 to +10 relative to the branch point, and −20 to 0 relative to the 3′ss are plotted, binning counts for tails of only adenosines (A_n, teal) separately from those of all other tails (other, purple). For A_n tails that map to the 3′-terminal nucleotide of introns, the fraction with each indicated length is plotted (right). The relative abundance of A_n at positions −2 and −1 relative to the 3′ss was ambiguous because most introns have an adenosine at position −1 (3′ss consensus sequence, YAG), which causes tails of length n at position −1 to be indistinguishable from tails of length n + 1 at position −2.
Extended Data Fig. 4 | RNA-seq profiles of genes with stable introns.
Profiles from the stable-intron-inducing condition (red) and log-phase culture (blue) are shown for the indicated 32 genes with confidently identified stable introns not already depicted in Fig. 1. For all but four of these, the profile of the stable-intron-inducing condition is from RNA-seq of the saturated-liquid sample. The four exceptions were not confidently identified in saturated liquid (Extended Data Table 2); for these, the profile of the stable-intron-inducing condition is from either the rapamycin-treated sample (TDA5 and YOS1) or the saturated lawn (RPO26 and RPS8B). Scale bars, 100 nucleotides. Otherwise, this panel is as in Fig. 1a.
Extended Data Fig. 5 | Stable introns are indistinguishable from other introns in many respects. a, Similar splicing motifs compared to other introns. Information-content logos of splicing motifs (6-mer 5′SS, 8-mer branch point and 3-mer 3′SS) for stable introns (bottom) and other introns (top) are plotted. b, Similar length distribution compared to other introns. Cumulative distributions of intron lengths are plotted (P = 0.057, two-tailed Kolmogorov–Smirnov test). c, No significantly enriched motifs within stable introns. Stable-intron sequence motifs discovered by MEME53 are plotted, either controlling for k-mer frequencies of non-stable introns (top) or without controlling (bottom). No significant motifs were discovered in stable introns when k ≥ 6. The motifs discovered without controlling for k-mer frequencies matched the canonical branch point and 5′SS motifs (see a). Branch point and 5′SS motifs were also the only significantly enriched motifs discovered when k ≤ 5. d, No enriched positional k-mer motifs detected by k-Logo54 in stable introns. The most enriched k-mers at positions relative to 5′SS (top), branch point (middle), and 3′SS (bottom) are plotted, comparing stable introns to unstable introns. Stacked nucleotides at a position represent the most significant motif starting at that position. The height is scaled relative to the significance of the motif, as determined by the one-sided binomial test statistic (y axes). Black numbers indicate invariant nucleotides occurring >95% of the time at the position. No k-mers were significantly enriched when using a Bonferroni-corrected P of 0.01. e, Support for a role of branch point–3′SS distance in specifying stable-intron formation. The indicated introns were ectopically expressed from the URA3 splicing construct (Extended Data Fig. 2b). Results are shown from an RNA blot that resolved total RNA from cultures overexpressing the indicated introns and was probed for the indicated introns (length of excised intron in parentheses). The ECM33 and RPL27B introns were probed sequentially, and then the ACT1, HNT2 and APS3 introns were probed concurrently. ACT1 probe was validated on synthetic transcripts resembling the ACT1 intron, which were produced by in vitro transcription (not shown). Migration of markers with lengths (in nucleotides) indicated is at the left. n ≥ 2 biological replicates. f, Expression of mRNAs from genes containing stable introns. Left, relationship between the expression in log-phase and saturated cultures (expression cutoff, 1 TPM in both samples). Points for genes containing stable introns are indicated (orange), and the point for ECM33 is labelled. Right, comparison of the expression results of genes with stable introns to those of the remaining genes. The cumulative distributions of fold changes in expression (expressed as log2(mRNA in log-phase culture/mRNA in saturated culture)) observed between log-phase and saturated cultures are plotted, which shows no significant difference between stable-intron genes and other genes (P = 0.31 two-tailed Kolmogorov–Smirnov test).
Extended Data Fig. 6 | Assessing aspects of TORC1 regulation on stable-intron expression. a, Inability of TORC1-independent inhibition of protein synthesis to prematurely induce stable-intron accumulation. The left lanes show a replicate of Fig. 3b, and the right lanes show results after treatment with either low (0.25 mg l\(^{-1}\)) or high (25 mg l\(^{-1}\)) concentrations of cycloheximide. As indicated by OD\(_{600}\) nm at collection, the mild cycloheximide treatment allowed the culture to reach an OD\(_{600}\) nm of 3.4 after 24 h, which was equivalent to the OD\(_{600}\) nm of 10 h without cycloheximide (Fig. 3a). b, Dispensability of TORC1-responsive Tap42 for stable-intron formation. Samples were grown at 25 °C owing to temperature sensitivity of the \(tap42-11\) allele. Additionally, owing to slower growth at 25 °C, the duration of pre-growth was extended to 5.5 h before reseeding in medium with (+) or without (−) rapamycin, such that the change in OD\(_{600}\) nm of the pre-growth sample matched that of wild-type cultures grown at 30 °C for 4 h. Otherwise, this panel is as in Fig. 3b. \(n = 2\) biological replicates. c, Dispensability of TORC1-sensitive Sch9 for stable-intron formation. Samples were grown in SC–Trp medium to maintain plasmids that expressed either TORC1-sensitive (pSCH9) or TORC1-insensitive (pSCH9DE) Sch9 protein. Because of the slower growth of the \(sch9\Delta pRS414::SCH9\) and \(sch9\Delta pRS414::SCH9DE\) strains (possibly owing to the requisite SC–Trp medium) the duration of pre-growth was extended to 5 h, such that the change in OD\(_{600}\) nm of the pre-growth sample matched that of wild-type cultures grown for 4 h. This panel is as in Fig. 3b. \(n = 3\) biological replicates. d, Dispensability of Sch9 for stable-intron formation. The left lanes show a replicate of Fig. 3b, and the right lanes show the same experiments performed in the \(sch9\Delta\) strain. \(n = 2\) biological replicates (\(sch9\Delta\) samples). e, Inability of acute deprivation of select nutrients to induce accumulation of stable introns. To prevent contamination of starting cultures with stable introns contributed by inoculum, cultures were seeded at OD\(_{600}\) nm = 0.2 from an overnight culture that was allowed to grow to mid-log phase, collected by vacuum filtration, washed in water and resuspended in fresh medium that lacked the indicated nutrients. Cultures were collected after the indicated times and analysed as in Fig. 1c, except the RNA blot was sequentially re-probed for the ECM33, SAC6 and USV1 introns. As indicated by OD\(_{600}\) nm at collection, the sample deprived of ammonium sulfate (the main source of nitrogen) was still able to reach a high density after 24 h. \(n \geq 2\) biological replicates. f, Inability of the general amino acid control response to induce stable introns. Early-log-phase cultures were treated with either 100 nM rapamycin or 50 mM 3-aminotriazole for the indicated times. Cultures were grown in SC–His to force inhibition of histidine biosynthesis by 3-aminotriazole. Cultures treated with 3-aminotriazole for 24 h did not reach a density sufficient to induce stable introns. Early-log-phase cultures were treated with either 100 nM rapamycin or 50 mM 3-aminotriazole for the indicated times. Cultures were grown in SC–His to force inhibition of histidine biosynthesis by 3-aminotriazole. Cultures treated with 3-aminotriazole for 24 h did not reach a density sufficient to induce stable introns. Approaching saturated growth. g, Stable-intron accumulation despite inability to induce the general amino acid control pathway. The left lanes show a replicate of Fig. 3b, and the middle and right lanes show the same experiments performed in \(gcn2\Delta\) and \(gcn4\Δ\) strains, respectively.
Extended Data Fig. 7 | The influence of hyperactive TORC1 on stable-intron formation. 

**a.** The left and right panels are biological replicates comparing ECM33 and SAC6 intron levels in a strain with hyperactive TORC1 (TORC1*) to those in a strain-background control (cdc28-4). Growth conditions were as described for these strains31. Overexpression of Sfp1 was induced 1 h before treatment with rapamycin (or no treatment). Owing to the decreased growth rate of these strains in the requisite conditions, samples with and without rapamycin were collected after 6 h of treatment rather than after 4 h of treatment. Numbers below the ECM33 and SAC6 intron blots indicate the level of each intron normalized to the level in the cdc28-4 rapamycin-treated samples, after first normalizing all lanes to the 5.8S rRNA loading control (set to 1).

**b.** Accumulation of the ECM33 intron in rapamycin-treated, saturated-lawn and saturated-liquid cultures, detected using RNA-seq. Results showing accumulation of this intron in saturated-liquid culture but not log-phase culture are from a different biological replicate than that shown in Fig. 1a, b; otherwise, this panel is as in Fig. 1a. Rapamycin results are re-plotted from Fig. 3d (4 h).

**c.** Overlap between stable introns identified in saturated-liquid, saturated-lawn and rapamycin-treated cultures.
Extended Data Fig. 8 | Evidence for spliceosome sequestration and control of ribosome production by stable introns. a, No evidence for compensatory stable-intron expression after genomic deletion of five stable introns. The scatter plot shows the relationship between intron accumulation in rapamycin-treated, wild-type culture and in rapamycin-treated, EUHSR culture, focusing on the 25 stable introns detected after rapamycin treatment (Extended Data Table 2). The dashed line is placed at $x = y$. Stable introns are indicated (closed orange circles). Points for introns deleted from the EUHSR genome (ECM33, UBC4, HNT1, SAC6 and RFA2) are labelled. All introns were pseudocounted at 0.5 TPM.

b, Aggregate stable-intron accumulation approaching that of spliceosomal RNAs. Levels of stable introns and spliceosomal RNAs (labelled closed blue circles) are plotted, comparing levels in log-phase, wild-type culture to those in rapamycin-treated, wild-type culture. The aggregate stable-intron abundance (closed orange circle, stable intron total) is also plotted. Much of the apparent accumulation observed in log-phase culture was attributable to reads from a few highly transcribed stable-intron genes (for example, RPS29A, which has an intron:mRNA TPM ratio of 107:2,030 in log phase compared to 289:319 in saturated culture). Otherwise, as in a.

c, Reduced expression of mRNA of ribosomal protein genes (RPGs) when overexpressing a stable intron in rapamycin-treated EUHSR culture. The cumulative distributions of fold changes in mRNA expression (expressed as log$_2$(mRNA in EUHSR expressing ECM33 intron/mRNA in EUHSR expressing ACT1 intron)) observed between an EUHSR culture with stable-intron (ECM33) ectopic expression and an EUHSR culture with control-intron (ACT1) ectopic expression are plotted. The distribution of changes for mRNAs of RPGs (green) differed from that of other genes (black), with generally lower expression of RPGs in the culture that overexpresses the stable intron ($P = 8.8 \times 10^{-32}$, one-tailed Kolmogorov–Smirnov test; expression cutoff, 1 TPM in both samples). d, Less efficient splicing—as detected by increased intron retention—when overexpressing a stable intron in rapamycin-treated, EUHSR culture. When analysing the dataset in c, 103 genes had significantly more intron retention when ectopically expressing the stable intron compared to when expressing the control intron (MISO, Bayes factor $> 100$). The Venn diagram shows the overlap between these genes with increased intron retention and intron-containing RPGs ($P = 3.9 \times 10^{-22}$, hypergeometric test).
Results are shown for the ten proteins that were consistently enriched ≥ 2-fold in stable-intron pull-down eluates. Three control samples without a tagged intron and three test samples with unique tagged introns (Extended Data Fig. 2, Supplementary Table 5) were simultaneously analysed by quantitative mass spectrometry. These ten proteins were the only proteins that were enriched ≥ 2-fold in each of the 9 possible pairwise comparisons between test and control samples. The identities of these proteins were consistent with the excised and debranched introns being part of a complex that resembles the ILS complex, in that all ten are known components of the ILS identified through biochemical studies23, and most (all but Brr2, Hsh49, Prp9 and Rse1) have also been identified in a cryo-EM structure of the ILS complex59. Moreover, several early spliceosome components (Luc7, Prp3, Snp1 and Snu13) as well as an essential disassembly factor (Prp43) were detected in all samples but not enriched in tagged-intron eluates. The observation that the intron did not co-purify with exonic sequences also helped to rule out the possibility that it was part of a complex that resembled earlier spliceosomal complexes. Enrichment values were those reported by PEAKS Studio, which are reported relative to values of control 2.

| Protein name | Control 1 | Control 2 | Control 3 | Test 1  | Test 2  | Test 3  | Mean control | Mean test | Fold difference |
|--------------|-----------|-----------|-----------|---------|---------|---------|--------------|-----------|-----------------|
| Prp9         | 0.11      | 1.00      | 0.57      | 4.84    | 6.34    | 4.83    | 0.56         | 5.34     | 9.54            |
| Hsh49        | 0.60      | 1.00      | 1.20      | 5.67    | 5.15    | 5.24    | 0.93         | 5.35     | 5.74            |
| Cef1         | 0.93      | 1.00      | 0.99      | 3.45    | 6.04    | 5.79    | 0.97         | 5.09     | 5.23            |
| Rse1         | 0.66      | 1.00      | 0.90      | 3.17    | 4.16    | 5.80    | 0.85         | 4.38     | 5.13            |
| Bud31        | 0.68      | 1.00      | 0.96      | 3.96    | 3.96    | 4.16    | 0.88         | 4.03     | 4.58            |
| Prp45        | 0.88      | 1.00      | 0.71      | 3.12    | 3.77    | 3.86    | 0.86         | 3.58     | 4.15            |
| Prp19        | 0.87      | 1.00      | 1.05      | 3.28    | 4.03    | 4.20    | 0.97         | 3.84     | 3.94            |
| Brr2         | 0.78      | 1.00      | 0.69      | 2.22    | 3.21    | 3.66    | 0.82         | 3.03     | 3.68            |
| Prp8         | 0.75      | 1.00      | 0.73      | 2.00    | 2.63    | 3.46    | 0.83         | 2.70     | 3.26            |
| Smb1         | 0.67      | 1.00      | 1.10      | 2.49    | 2.80    | 2.69    | 0.92         | 2.66     | 2.88            |
| Stable intron | Liquid | Rapamycin | Lawn | BP-3'SS distance (nt) | Intron length (nt) | Description of gene function |
|--------------|--------|-----------|------|-----------------------|--------------------|-------------------------------|
| AIM11        | ●      | ●         | ●    | 20                    | 75                 | Protein of unknown function  |
| ARF2         | ●      | ●         | ●    | 18                    | 332                | ADP-ribosylation factor      |
| ARP2         | ●      | ●         | ●    | 15                    | 123                | Essential component of the Arp2/3 complex |
| CMC2         | ●      | ●         | ●    | 29                    | 85                 | Protein involved in respiratory chain complex assembly or maintenance |
| CNB1         | ●      | ●         | ●    | 19                    | 76                 | Calcineurin B                 |
| DID4         | ●      | ●         | ●    | 23                    | 68                 | Class E Vps protein of the ESCRT-III complex |
| DYN2_2       | ●      | ●         | ●    | 17                    | 80                 | Cytoplasmic light chain dynein, microtubule motor protein |
| ECM33        | ●      | ●         | ●    | 25                    | 330                | GPI-anchored protein of unknown function |
| EFMS_2       | ●      | ●         | ●    | 25                    | 93                 | S-adenosylmethionine-dependent lysine methyltransferase |
| GLC7         | ●      | ●         | ●    | 31                    | 525                | Type 1 S/T protein phosphatase (PP1) catalytic subunit |
| GPI15        | ●      | ●         | ●    | 14                    | 74                 | Protein involved in the synthesis of GlcNac-PI |
| HNT1         | ●      | ●         | ●    | 23                    | 111                | Adenosine S'-monophosphoramidase |
| KEI1         | ●      | ●         | ●    | 31                    | 101                | Component of inositol phosphoceramide (IPC) synthase |
| MPT5         | ●      | ●         | ●    | 25                    | 640                | mRNA-binding protein of the PUF family |
| NBL1         | ●      | ●         | ●    | 12                    | 67                 | Subunit of the conserved chromosomal passenger complex (CPC) |
| POP8         | ●      | ●         | ●    | 20                    | 75                 | Subunit of both RNase MRP and nuclear RNase P |
| QCR10        | ●      | ●         | ●    | 15                    | 63                 | Subunit of the ubiquinol-cytochrome c oxidoreductase complex |
| RFA2         | ●      | ●         | ●    | 24                    | 108                | Subunit of heterotrimeric Replication Protein A (RPA) |
| RIM1         | ●      | ●         | ●    | 26                    | 83                 | ssDNA-binding protein essential for mitochondrial genome maintenance |
| RPO26        | ●      | ●         | ●    | 21                    | 76                 | RNA polymerase subunit ABC23 |
| RPS23A       | ●      | ●         | ●    | 23                    | 319                | Ribosomal protein 28 (rp28) of the small (40S) ribosomal subunit |
| RPS29A       | ●      | ●         | ●    | 22                    | 488                | Protein component of the small (40S) ribosomal subunit |
| RPS6B        | ●      | ●         | ●    | 23                    | 352                | Protein component of the small (40S) ribosomal subunit |
| RPS8B        | ●      | ●         | ●    | 24                    | 360                | Protein component of the small (40S) ribosomal subunit |
| SAC6         | ●      | ●         | ●    | 20                    | 111                | Fimbrin, actin-bundling protein |
| SAR1         | ●      | ●         | ●    | 19                    | 139                | ARF family GTPase |
| SUN4         | ●      | ●         | ●    | 22                    | 346                | Cell wall protein related to glucanases |
| TDA5         | ●      | ●         | ●    | 19                    | 71                 | Protein of unknown function |
| UBC4         | ●      | ●         | ●    | 26                    | 95                 | Ubiquitin-conjugating enzyme (E2) |
| URA2         | ●      | ●         | ●    | 22                    | 320                | Bifunctional carbamoyl/phosphate synthetase/aspartate transcarbamylase |
| USV1         | ●      | ●         | ●    | 24                    | 75                 | Putative transcription factor containing a C2H2 zinc finger |
| VMA9_1       | ●      | ●         | ●    | 22                    | 77                 | Vacuolar H+ ATPase subunit e of the V-ATPase V0 subcomplex |
| YCL012C      | ●      | ●         | ●    | 13                    | 65                 | Protein of unknown function |
| YOS1_2       | ●      | ●         | ●    | 24                    | 111                | Integral membrane protein required for ER to Golgi transport |

If a host gene has two introns, a suffix specifies whether the first or second intron is stabilized (_1 and _2, respectively). Description of gene function is from YeastMine (https://yeastmine.yeastgenome.org/). Liquid, saturated-liquid sample; lawn, saturated-lawn sample; rapamycin, rapamycin-treated sample.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

![Checkmarks indicating which items are confirmed.]

**n/a** Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

**Data collection** No software was used.

**Data analysis** RNA-blot data were analyzed with ImageQuant TL (v8.1.0.0). Growth curves were analyzed with SkanIt (v3.2). RNA-seq data were analyzed with cutadapt (v1.8), STAR (v2.4), and MISO (v0.5.4), and visualized with IGV (v2.3.57).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequencing data and the processed data for each gene and intron are available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE121765. For gel source data, see Supplementary Figure 1. All other data are available from upon reasonable request.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | In growth-curve experiments, technical replicates were used for each strain/condition combination, per standard practice. Variance observed between biological replicates showed that three were sufficient for growth curves and one was sufficient for RNA-seq. |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | In growth-curve experiments, single wells were excluded if artifactual spikes in absorbance attributable to bubbles or condensation were observed. Results for a replicate were excluded if more than one well was censored. |
| Replication | RNA-seq results for biological replicates correlated well (log-phase culture, Pearson R² = 0.98 [mRNA, n = 5652]; saturated culture, Pearson R² = 0.88 [mRNA, n = 5762] and 0.78 [stable introns, n = 30]). Growth curves were each performed using at least 3 biological replicates. |
| Randomization | Growth-curve cultures were randomized by permutation of strain placement on 96-well plates across experiments. |
| Blinding | LC/MS: peak selection and data analysis were done blindly by the staff of the metabolic profiling facility, without knowledge of conditions. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ | Unique biological materials |
| ☑ | Antibodies |
| ☑ | Eukaryotic cell lines |
| ☑ | Palaeontology |
| ☑ | Animals and other organisms |
| ☑ | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ | ChiP-seq |
| ☑ | Flow cytometry |
| ☑ | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials | Unique yeast strains and plasmids are available upon request from the authors and Addgene, respectively. |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | Yeast strains not unique to this study are common strains (BY4741, W303a), were requested from labs of origin per standard practice, or were obtained commercially (yeast knock out collection, Horizon Discovery). |
|-------------------|--------------------------------------------------------------------------------------------------|
| Authentication | ORF deletions, intron deletions, and BP manipulations were authenticated by amplification and Sanger sequencing across loci of interest. |
| Mycoplasma contamination | Because our eukaryotic cell lines were all yeasts, no mycoplasma testing was performed. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |