The folding of newly synthesized proteins to the native state is a major challenge within the crowded cellular environment, as non-productive interactions can lead to misfolding, aggregation and degradation. Cells cope with this challenge by coupling synthesis with polypeptide folding and by using molecular chaperones to safeguard folding cotranslationally. However, although most of the cellular proteome forms oligomeric assemblies, little is known about the final step of folding: the assembly of polypeptides into complexes. In prokaryotes, a proof-of-concept study showed that the assembly of heterodimeric luciferase is an organized cotranslational process that is facilitated by spatially confined translation of the subunits encoded on a polycistrionic mRNA. In eukaryotes, however, fundamental differences—such as the rarity of polycistrionic mRNAs and different chaperone constellations—raise the question of whether assembly is also coordinated with translation. Here we provide a systematic and mechanistic analysis of the assembly of protein complexes in eukaryotes using ribosome profiling. We determined the in vivo interactions of the nascent subunits from twelve hetero-oligomeric protein complexes of *Saccharomyces cerevisiae* at near-residue resolution. We find nine complexes assemble cotranslationally; the three complexes that do not show cotranslational interactions are regulated by dedicated assembly chaperones. Cotranslational assembly often occurs unidirectionally, with one fully synthesized subunit engaging its nascent partner subunit, thereby counteracting its propensity for aggregation. The onset of cotranslational subunit association coincides directly with the full exposure of the nascent interaction domain at the ribosomal tunnel exit. The action of the ribosome-associated Hsp70 chaperone Ssb is coordinated with assembly, Ssb transiently engages partially synthesized interaction domains and then dissociates before the onset of partner subunit association, presumably to prevent premature assembly interactions. Our study shows that cotranslational subunit association is a prevalent mechanism for the assembly of hetero-oligomers in yeast and indicates that translation, folding and the assembly of protein complexes are integrated processes in eukaryotes.

To test whether protein assembly in eukaryotes initiates during translation, we analysed 12 hetero-oligomeric complexes of *S. cerevisiae* (Extended Data Table 1). They were chosen to represent a variety of cellular functions, structural architectures, regulatory features, abundance and interface size. They are all verified complexes, mainly stable ones, with surface-exposed C termini for affinity tagging, and cytoplasmic or nuclear localization.

To identify the nascent-chain interaction profiles of complex subunits in vivo, we used selective ribosome profiling (SeRP). SeRP compares the distribution of ribosome-protected mRNA footprints of two distinct samples generated from a single culture. One comprises the ribosome protected footprints of all translated open reading frames (ORFs) (total translatome). The other contains footprints of a selected set of ribosomes, co-purified with a tagged interaction partner (selected translatome). Accumulation of footprints in the selected translatome, as compared to the total translatome, directly indicates when it is during translation that the nascent chain interacts with the affinity-purified tagged protein subunit, at near-residue resolution.

We first analysed the assembly of fatty acid synthase (FAS), a multi-functional enzyme integrating all the fatty acid biosynthesis steps. FAS is composed of two multi-domain subunits, α and β, which assemble to a highly intertwined, 2.6-MDa, hetero-dodecameric (α6β6) complex. (Fig. 1a, d). To capture cotranslational assembly in vivo, we generated two strains, each chromosomally encoding one of the FAS subunits C-terminally fused to GFP for immunopurification. Tagging did not affect function (Extended Data Fig. 1a). SeRP demonstrates that FAS assembly initiates cotranslationally in a specific, asymmetric manner. Tagged α does not engage ribosome–nascent-chain complexes (RNCs) translating α or β. By contrast, tagged β engages RNCs synthesizing nascent α, leading to a strong, approximately 40-fold enrichment of selected footprints over total ribosome-protected footprints, starting near residue 125 of α and persisting until synthesis ends (Fig. 1b). This asymmetry of cotranslational interactions contrasts with immunoblotting results for the mature FAS, showing that each FAS subunit can immunopurify their partner subunit post-translationally with the same 1:1 stoichiometry (Extended Data Fig. 1b). The FAS subunits hence have distinct roles in the cotranslational assembly of the complex.

The onset of cotranslational subunit engagement directly correlates with FAS structural features: it coincides with ribosome exposure of the first 94 amino acids of α—which are intertwined with the last 389 amino acids of β—to form a single catalytic domain, the malonyl-palmitoyl-transferase (MPT) domain. This implies that cotranslational assembly initiates upon formation of the MPT domain, the most stable interface between the two subunits. To test whether the MPT interface is indeed required for cotranslational assembly of FAS, we analysed cotranslational interactions of FAS-deletion mutants lacking the MPT segments. Supporting the proposed model, MPT segments deletion, in either α or β, strongly reduces cotranslational interactions (Fig. 1c).

We tested whether cotranslational interactions are nascent-chain dependent by puromycin treatment, triggering the release of nascent chains from ribosomes. Quantitative reverse transcription PCR (RT-qPCR) after immunopurification of the β subunit revealed that puromycin reduces the level of co-purified α-encoding mRNAs (Extended Data Fig. 1c, d), suggesting that cotranslational assembly relies on subunit association with nascent chains during translation. We next tested the extent of post-lysis association of β with nascent α and found it to be very low (Extended Data Fig. 1e–g). We conclude our SeRP setup provides snapshots of physiological interactions with RNCs that were established in vivo. Taken together, our findings indicate that the assembly of the dodecameric (α6β6) FAS initiates cotranslationally by the formation of αβ hetero-dimers, mediated by the interaction of the C terminus of β with the N terminus of nascent α to form the MPT domain (Extended Data Fig. 1h).

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folding and assembly of the unstable α, protecting it from aggregation. Thus, cotranslational assembly may ameliorate the challenging folding trajectory of α.

We next analysed the assembly of a hetero-trimeric complex, the multi-aminoacyl-tRNA synthetase. This complex is composed of the essential methionyl- and glutamyl-tRNA synthetases MetRS and GluRS (encoded by MES1 and GUS1, respectively), both of which are required for charging their specific tRNA with cognate amino acids, and the Arc1p cofactor, which regulates their catalytic activities and subcellular distributions 18–20 (Fig. 2a, d). We generated three strains, each chromosomally encoding one of the complex subunits C-terminally fused to GFP. Tagging did not affect function (Extended Data Fig. 2a). SeRP revealed that both GluRS and MetRS engage each other cotranslationally, resulting in at least a 30-fold enrichment in footprints, starting at codons 196 and 168 of GUS1 and MES1, respectively, and persisting until synthesis ends. Both catalytic subunits also engage the nascent Arc1p cofactor, with nearly identical onsets approximately at codon 160 of ARCI (Fig. 2b). For all these nascent chains, the onset of partner subunit engagement occurs upon emergence at the ribosome exit tunnel of the N-terminal interaction domains, which share a similar glutathione-S-transferase (GST)-like fold 20. Either catalytic subunit can thus cotranslationally engage all other subunits. By contrast, the fully synthesized Arc1p associates mainly with nascent GluRS (starting at codon 143) in a fluctuating manner, suggesting that these interactions are less stable compared to the cotranslational interactions of each of its subunits in a network-like manner (Extended Data Fig. 2b), which involves the shared GST-like folds acting as assembly drivers.

Notably, both GluRS and MetRS are bi-functional proteins regulating ATP-synthase expression upon glucose depletion. Arc1p is then rapidly degraded; MetRS relocates to the nucleus and GluRS relocates to the mitochondria 21. As the localization signal of each of the two subunits is buried within the interface domains upon trimerization 21, we speculate that cotranslational assembly can regulate dual protein targeting in eukaryotes, by prioritizing cytosolic activity under favourable growth conditions.

To investigate the prevalence of the cotranslational assembly mechanism, we subjected 10 additional complexes to SeRP analysis. In total, 12 complexes composed of 26 individual subunits were analysed. We find that 9 out of 12 complexes exhibit cotranslational subunit interactions, demonstrating the prevalence of this assembly mechanism among stable cytosolic complexes (see PFK, TRP further examples in Extended Data Figs. 3, 4; Extended Data Table 2). Six out of nine complexes use a directional assembly mode, with one specific subunit being released from the ribosome before engaging the nascent interaction partner or partners (FAS, NatA, NatB, TRP, CPA and eIF2; Extended Data Table 2).

We hypothesized the cotranslationally engaged subunits have a higher propensity to misfold compared to their fully-synthesized partners. Accordingly, FAS subunits display asymmetric misfolding propensities 14–17. To test whether this is a general feature, we performed in vivo aggregation and stability assays of subunits in wild-type and single subunit deletion strains for NatA, TRP and CPA. We excluded all complexes that are essential (eIF2) 22 or show severe growth phenotype upon subunit deletion (NatB) 23. All nascently engaged subunits tested are indeed prone to aggregation or degradation in the absence of their partner subunits. By contrast, subunits that are only engaged after release from the ribosome are much more soluble and stable in the absence of their partner subunits (Extended Data Fig. 5a–c). Our findings suggest that in particular aggregation-prone subunits engage their partner subunits cotranslationally.

Three complexes do not show cotranslational assembly: (i) 20S proteasome subunits α1, 2; (ii) V-type-ATPase catalytic hexamer (A3, B3) and (iii) ribonucleotide reductase RNR (Rrn2p and Rrn4p complex). All three complexes are tightly controlled by dedicated
The position-resolved cotranslational interaction profiles of all 14 subunits identified in this study enabled us to reveal general features of the assembly process. We find that the onsets of interactions vary, but they are generally stable, persisting until synthesis ends (Fig. 3a, Extended Data Fig. 5d). Analysis of the nascent-chain features revealed that subunits containing extreme C-terminal interaction domains are excluded. In nearly all complexes, subunits are engaged when a complete interaction domain and an additional 24–37 amino acids have been synthesized (Fig. 3b). The eukaryotic ribosomal tunnel accommodates approximately 24 amino acids in extended conformation and approximately 38 amino acids in α-helical conformation. Thus, the sharp onset of assembly (Fig. 3c) directly correlates with the emergence of the entire interface domain from the ribosome exit tunnel. Taken together, our results suggest assembly is facilitated by interface domains cotranslational folding.

Folding of nascent polypeptides in yeast is facilitated by the Hsp70 family member Ssb, the major ribosome-associated chaperone. Ssb is targeted to the ribosome by the RAC complex and by direct contacts with the exit tunnel, ensuring high affinity to short, hydrophobic nascent-chain segments. This raises the question of how Ssb binding relates to cotranslational complex assembly. Analysis of Ssb SeRP interaction profiles shows that all nascent chains that engage partner subunits have one or multiple Ssb binding peaks. Ssb binds 13 out of 14 subunits before the onset of cotranslational assembly, generally during the synthesis of interaction domains, and dissociates just before subunit engagement (Fig. 4b, c for examples; Fig. 4d, e). Ssb engagement is thus well coordinated with assembly. We propose that Ssb shields hydrophobic patches within interaction domains, protecting them from non-productive interactions and misfolding. Ssb dissociates upon full ribosome exposure of these domains, permitting cotranslational folding and subunit joining. We further investigated Ssb interplay with assembly by a proteome-wide bioinformatics analysis, identifying all putative cotranslationally assembled subunits (for details, see ‘Data analysis section’ in Methods). Metagene profiling of Ssb binding to these ORFs and/or nascent chains demonstrates that Ssb generally dissociates just before putative cotranslational assembly–onset positions, which are characterized by low hydrophobicity (Extended Data Fig. 6). We suggest that the low hydrophobicity disfavours Ssb binding, allowing for interface domain folding and subunit interaction (see conclusions for model). To directly assess the effect of Ssb on cotranslational assembly, we attempted SeRP experiments in ssb1∆ssb2A mutants. However, these experiments repeatedly failed owing to the low amounts of ribosomes co-purified with tagged subunits. Nevertheless, these results are consistent with Ssb having an important role in cotranslational assembly. Accordingly, ssb1∆ssb2A mutants display widespread aggregation of newly synthesized proteins, among which complex subunits are enriched—including most of the complex subunits analysed here (Extended Data Table 2).

Beyond complex assembly, we hypothesized that cotranslational interactions may extend to all protein–protein networks. We tested this possibility by identifying the proteome-wide nascent-chain interactions of some subunits in our dataset, focusing on the subunits of enzymatic pathways. We adapted a recently developed peak detection algorithm, to identify local binding peaks, which were defined as a greater than threefold enrichment in footprint density over a stretch of more than ten codons. For FAS β, PFK β and Cpa2 subunits we detected additional, transient interactions with distinct sets of RNCs known to be functionally related to or directly interacting with the subunit (examples in Extended Data Fig. 7). One example is FAS β, which engages nascent acetyl-CoA carboxylase (Acc1p). Acc1p catalyses the step directly preceding FAS in the pathway (Extended Data Fig. 7a). Unlike the stable engagement of FAS β with nascent α for assembly, its association with nascent Acc1p is transient, similar to the interactions between fully synthesized FAS and Acc1p that have previously been reported. Nonetheless, it is specific, as β does not engage any other nascent member of the fatty acid synthesis pathway (Extended Data Fig. 7a). These findings provide first evidence that metabolic pathways can be coordinated cotranslationally. The extent and function of such nascent-chain interactomes have yet to be revealed.

To conclude, our study provides direct in vivo evidence, at near-residue resolution, that cotranslational subunit engagement is a widespread mechanism for complex assembly in eukaryotes. Our findings are consistent with previous studies that used indirect approaches to study cotranslational interactions in eukaryotes, such as RNA-IP-microarray (RIP-Chip) or in vitro translation system.
The high misfolding propensities of the subunits that interact as nascent chains with partner subunits underscore the importance of this mechanism. Cotranslational assembly may be a prerequisite for the evolvement of complex folding architectures and the rescue subunits destabilized by accumulating mutations. We furthermore reveal an intricate functional interplay between the Ssb chaperone and complex assembly onset.

Fig. 3 | Characteristics of cotranslational complex assembly interactions. a, Onset and persistence of the cotranslational interaction of each subunit with its partner complex subunit or subunits, for all 14 subunits identified as cotranslationally engaged. NAA20 and NAA15 are also known as NAT3 and NAT1, respectively. b, Top, interaction domain exposure correlated to the onset of assembly onset. Bottom, an expanded view of the region surrounding the onset of assembly. c, Normalized mean read density of interaction profiles of 14 cotranslationally engaged subunits, aligned and zoomed-in to the onset of assembly of each nascent chain. AU, arbitrary units.

Fig. 4 | Coordination of cotranslational complex assembly with the ribosome-associated chaperone Ssb binding. a, Illustration of ribosome-nascent-chain binding to Ssb or a partner subunit. b, c, Zoomed-in interaction profiles of Ssb1–GFP and cotranslationally engaged partner subunits with the nascent FAS and nascent GluRS, respectively. The area between replicates is shaded, indicating the degree of experimental variation. d, Heat map of Ssb1–GFP binding to ribosomes synthesizing the 14 cotranslationally engaged subunits, compared to complex assembly onset. e, Metagene analysis of the Ssb1–GFP interaction profiles with 14 cotranslationally engaged nascent chains, aligned and zoomed-in to assembly onset, compared to random position along the ORFs alignment. There is no correlation between the onset and random position alignment (Pearson correlation $r = 0.01256$), thus Ssb depletion at onset positions is significant. The area between replicates is shaded, indicating the degree of experimental variation. b–e, Data are from two biologically independent experiments.
the binding of partner subunits, suggesting that nascent subunits are constantly engaged (for model, see Extended Data Fig. 8). Conversely, exposed interfaces may serve as signals for subunit degradation, providing a molecular basis for quality control and the regulation of subunit stoichiometry at the level of the nascent chain. We further speculate that the translation of complex subunits is spatially confined in the cytosol, as this would facilitate timely assembly and prevent prolonged nascent-chain exposure.

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/s41586-018-0462-y

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### METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

#### Strains construction

GFP-tagged strains and deletion strains were generated via homologous recombination, constructed according to previously published work. For the GFP tag, a cassette containing the monomeric GFP gene and a G418 resistance marker was amplified from the pYEp12-mGFP plasmid. For gene deletions, a cassette containing only a selection marker was PCR amplified. All experiments were performed in the BY4741 strain background. *S. cerevisiae* strains used in this study are listed in Supplementary Table 1.

#### Yeast cultures

Yeast cultures were cultivated either in liquid yeast extract–peptone–dextrose (YPD)-rich medium, or in synthetic dextrose (SD) minimal medium (1.7 g/l yeast nitrogen base with ammonium sulfate or 1.7 g/l yeast nitrogen base without ammonium sulfate with 1 g/l monosodium glutamic acid, 2% glucose and supplemented with a complete or appropriate mixture of amino acids) at 30°C. Trp2-GFP, Trp3-GFP strains were grown in SD lacking tryptophan; and Cpa1-GFP, Cpa2-GFP were grown in SD lacking arginine, to induce their expression. For fatty acid supplementation, SD media was supplemented with 0.03% Myristic acid (Sigma, pre-dissolved in DMSO), 0.1% TWEEN-40 (Sigma), and 0.05% yeast extract.

#### Purification of RNAs for SeRP

Approximately 800 ml of cell culture was grown to an OD_{600} nm of 0.5, at 30°C, in appropriate medium. Cell collection was performed in the culture medium as follows: cells were collected rapidly by vacuum filtration on 0.45-µm nitrocellulose (Aamershing) blotting membrane and then flash frozen, as previously described. Next, cells were lysed by cryogenic grinding in a mixer mill (2 min, 30 Hz, MM400 Retsch) with 900 µl of lysis buffer (20 mM Tris–HCl pH 8.0, 140 mM KCl, 6 mM MgCl₂, 1.0% NP-40, 0.1 mg/ml cycloheximide (CHX), 1 mM PMSF, 2 × protease inhibitors (Complete EDTA-free, Roche), 0.02 U/ml RNase (recombinant RNase A, Roche), 20 mg/ml leupeptin, 20 mg/ml aprotinin, 10 mg/ml E-64, 40 µg/ml bestatin). Lysates were centrifuged (2 min at 30,000g, 4°C).

For each experiment, supernatants were divided for total (200 µl) and immunopurification (700 µl) translate samples. Total samples were digested using 10 U per A260 nm unit of RNaseA for 25 min at 4°C, in rotation, then loaded onto 800 µl of sucrose cushions (25% sucrose, 20 mM Tris–HCl pH 8.0, 140 mM KCl, 10 mM MgCl₂, 0.1 mg/ml CHX, 1 × protease inhibitors) and centrifuged in a TLA120 rotor for 90 min at 75,000 × g, 4°C. Pellets were resuspended in lysis buffer and transferred to non-stick tubes. 100–200 mg of total RNA were taken for ribosome profiling of the total translate.

Immunopurification samples were digested using 10 U per A260 nm unit of RNaseA, together with 100–400 µl of GFP-binder slurry and the suspension was rotated for 25 min, 4°C. Beads were washed three times in wash buffer 1 (20 mM Tris–HCl pH 8.0, 140 mM KCl, 10 mM MgCl₂, 0.1 mg/ml CHX, 1 × protease inhibitors) and centrifuged in a TLA120 rotor for 20 min at 15,000 × g, 4°C. Beads were resuspended in lysis buffer and centrifuged for 2 min at 30,000 × g, 4°C. For each experiment, supernatants were divided for total (200 µl) and immunopurification (700 µl) translate samples. Total samples were digested using 10 U per A260 nm unit of RNaseA for 25 min at 4°C, in rotation, then loaded onto 800 µl of sucrose cushions (25% sucrose, 20 mM Tris–HCl pH 8.0, 140 mM KCl, 10 mM MgCl₂, 0.1 mg/ml CHX, 1 × protease inhibitors) and centrifuged in a TLA120 rotor for 90 min at 75,000 × g, 4°C. Pellets were resuspended in lysis buffer and transferred to non-stick tubes. 100–200 mg of total RNA were taken for ribosome profiling of the total translate.

#### cDNA library preparation for deep sequencing

Library preparation was performed mainly as described in. In summary, RNA extraction was performed by mixing 0.75 ml pre-warmed acid phenol (Ambion) with either the purified monocistronic library for deep sequencing.

#### Ratio-based enrichment profiles analysis

The ratio-based enrichment profiles were built by comparing the RPM (reads per million mapped reads) interactome and translome data at each nucleotide along the ORFs. The reproducibility of replicates of interaction profiles was evaluated by Pearson correlation analysis. If a threshold of 0.6 was passed, genes were processed further.

To exclude genes that are expressed close to the background level or have a low read coverage, we defined minimal requirement thresholds that must be all passed before genes were considered for analysis: (i) at least 64 reads in both subunit-bound and total translateome datasets; (ii) at least 8 RPMK reads (reads per kilobase of transcript, per million mapped reads) in both translateome datasets; (iii) at least one position after the first 90 nucleotides in the subunit-bound translateome that has a twofold higher read number than the average of the first 90 nucleotides (designated 90 nucleotides background giving the specific background signal for every gene; for genes lacking any read in the 90 nucleotide background, the average read per nucleotide along the complete gene from the corresponding translateome is used).

#### Data analysis

Sequenced reads were processed as previously described using standard trimming and genome alignment tools (Cutadapt, Bowtie2, TopHat2) and Python scripts adapted to *S. cerevisiae*. SeRP analyses are based on at least two independent biological replicates that were highly reproducible, as evaluated by Pearson correlation analysis of each gene profile, see details below.

### Metagen analysis

For metagen analyses genes were normalized to their expression level by dividing the read density of each nucleotide by the average read density per nucleotide of the respective gene. Replicates reproducibility of gene profiles was evaluated by Pearson correlation analysis. If a threshold of 0.6 was passed, genes were processed further.
The genes were aligned to the position of onset of cotranslational complex assembly interactions, defined by the single codon position where the enrichment threshold (defined in the previous section) was crossed for each gene. Proteome-wide bioinformatics analysis of Ssb1 interplay with putative cotranslational complex assembly interactions:

To determine the proteome-wide interplay of Ssb SeRP interaction profiles with cotranslational assembly interactions, we performed a bioinformatics analysis to identify all hetero-oligomeric complexes subunits that are putatively cotranslationally assembling, and their putative assembly onset positions.

For the analysis we have used the following parameters, extrapolated from our experimental data: of all PDB-deposited structures of hetero-oligomeric complexes, we identified subunits containing N′ terminal protein interface domains, located in the first 40% of genes, as our experimental dataset is disenriched for subunits containing extreme C′-terminal ones. Complexes involving interface domains smaller than five interacting residues were removed, as our experimental dataset is disenriched for this type of complex. Complexes involving dedicated assembly chaperones/precursors were excluded, as our experimental dataset show this type of complex is less likely to cotranslationally assemble.

We next identified ends of N′ terminal interfaces as assembly onset positions, as our experimental data shows most assembly onsets occur directly upon the emergence of an entire interface domain from the ribosome exit tunnel.

We used a geometric clustering algorithm (http://www.blopig.com/blog/2013/10/get-pdb-intermolecular-protein-contacts-and-interface-residues/from-the-Oxford-Protein-Informatics-Group) to identify the patches of interface atoms within each subunit. Atoms directly involved in protein–protein interfaces were defined by an intermolecular distance cutoff of 4.5 Å, on the basis of previously published works33-35.

Interface patch atoms were defined by an intramolecular distance cutoff of 10 Å, according to their Cα distances within the crystal component, on the basis of previously published work33,34.

Interface patches size threshold. Patches must include at least five interacting residues. Structures with resolution greater than 10 Å were removed from the analysis, in accordance with these defined interface parameters, as the cutoff of 10 Å could not be determined. Membrane protein complexes were removed from the analysis. Structures of truncated proteins lacking their N′ terminal interfaces were removed from our analysis.

We aligned all proteins to the position of putative onset of cotranslational assembly interactions: to the ends of N′ terminal interfaces and performed a metagene profile of Ssb1 binding to this subset with Ssb1 SeRP experiments10.

ORFs with low Ssb1 SeRP footprint coverage were removed from the analysis, using a threshold of 64 total counts per ORF to maintain significant reproducibility between SeRP independent biological replicates36-37. The reproducibility of replicates of interaction profiles was evaluated by Pearson correlation analysis. If a threshold of 64 total counts per ORF was used for an appropriate dilution) was mixed with 7.5 μl reaction Master Mix (5 μl Flash SYBR Green Mix, 1.7 μl DEPC H2O, 0.4 μl primer (10 μM)) with a multistep pipette to reduce pipetting errors. For analysis the following program was used: pre-incubation: 95 °C, 5 min; amplification: 95 °C, 10 s, 55 °C, 20 s, 72 °C, 20 s; single acquisition mode; melting curve: 60–90 °C, 0.11 °C/second acquisition mode. Cq values were calculated by derivation by the LightCycler480 software (Roche). For normalization ACT1 mRNA was used as a housekeeping gene.

CHX chase and flow cytometry analysis. Yeast cells were grown to log phase, then CHX (10.5 μM) was added, and aliquots from each time point were taken. GFP levels of fixed cells at each time point were determined by flow cytometry analysis performed using a BD FACS Canto II equipped with lasers (405 nm, 488 nm, 635 nm). Detectors used: FSC, SSC, 488-E for GFP with filter 530/30. Cell population gated on FSC/SSC area dot plot, exclusion of debris and cell aggregates by SSC/FSC height and width. Twenty thousand events/cell/sample. Data are from three biologically independent experiments.

Quantification and statistical analysis. Blinding or randomization was not used in any of the experiments. The number of independent biological replicates used for an experiment is indicated in the respective figure legends. The statistical tests and P values used for the interpretation of data are mentioned in the figure legends.

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

Code availability. Customized Python scripts for data analysis are available from the corresponding authors upon request.

Data availability. The data supporting the findings of this study have been deposited in the Gene Expression Omnibus (GEO) repository with the accession code GSE116570. Figure 4 and Extended Data Fig. 6 also rely on raw data derived from the dataset of the Ssb1 SeRP experiments, which are available under accession code GSE93830. All other data are available from the corresponding authors upon reasonable request.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Functionality of GFP-tagged FAS complex subunits, characteristics of co- versus post-translational FAS subunit interactions and the FAS assembly model. a, GFP tagging of the FAS complex subunits does not affect growth under fatty acid depletion conditions, as compared to wild-type (YPD, right compared to YPD + fatty acids, left). A representative image from three biologically independent experiments is shown. b, Immunoblotting of the FAS complex subunits in input, flow through and immunopurification fractions of a typical SeRP experiment analysing samples of strains encoding either GFP-tagged $\alpha$ or $\beta$ subunits. Data are from three biologically independent experiments. c, Puromycin-induced release of nascent chains (10 $\mu$g/ml, 10 min post lysis) decreases the interaction of nascent $\alpha$ with the C-terminally tagged $\beta$ subunit, analysed by immunopurification followed by RT–qPCR. Data are normalized mean mRNA levels ± s.e.m. with each data point from three biologically independent experiments overlaid. d, Polysome profiles of samples following puromycin (puro) treatment (as in c) or CHX treatment. Data representative of three biologically independent experiments are shown. e, Post-lysis binding control: experimental scheme. Two independent cultures, of two strains, expressing either wild-type $\alpha$ subunit and C-terminally GFP-tagged $\beta$ subunit; or wild-type $\beta$ subunit and C-terminally TAP-tagged $\alpha$ subunit, were grown to log phase, $\text{OD}_{600}$ 0.5. The cells were then mixed in a 1:1 ratio and subsequently lysed, subjected to GFP immunopurification and SeRP. f, Predicted SeRP engagement of nascent wild-type $\alpha$ subunit or $\alpha$–TAP ORF, by C-terminally GFP-tagged $\beta$ subunit. No post-lysis interactions: no detection of ribosome protected footprints of mRNA encoding TAP (top). Post-lysis interactions: detection of ribosome protected footprints of TAP-encoding mRNA at a similar level to wild-type $\alpha$ subunit ORF (bottom). g, Results of post-lysis binding control: engagement of nascent wild-type $\alpha$ subunit or $\alpha$–TAP by C-terminally GFP-tagged $\beta$ subunit, analysed by SeRP, as in Fig. 1. Data are from two biologically independent experiments. h, Model of the FAS complex assembly pathway.
Extended Data Fig. 2 | Functionality of GFP-tagged multi-aminoacyl-tRNA synthetase complex subunits and the assembly model. a, GFP tagging of the essential multi-aminoacyl-tRNA synthetase complex subunits does not affect growth, as compared to wild type (YPD). b, Model of the multi-aminoacyl-tRNA synthetase complex assembly pathways.

A representative image from three biologically independent experiments is shown.
Extended Data Fig. 3 | Cotranslational assembly of the anthranilate synthase complex. a, Domain organization of the anthranilate synthase subunits. b, Engagement of nascent Trp2p (tryptophan 2) and Trp3p (tryptophan 3) by C-terminally-tagged Trp2p subunit (top) compared to engagement of nascent Trp2p and Trp3p by C-terminally-tagged Trp3p subunit (bottom), analysed by SeRP. Data are from two biologically independent experiments. Coloured numbers indicate ribosome positions where the enrichment stably crosses the twofold threshold. The area between replicates is shaded, indicating the degree of experimental variation. c, Crystal structure of the homologous anthranilate synthase complex from the archaea Sulfolobus solfataricus (~60% sequence similarity, PDB: 1QDL1). d, GFP tagging of the complex subunits does not affect cell growth under tryptophan depletion conditions (YPD, right panel compared to SD lacking tryptophan, left). A representative image from three biologically independent experiments is shown. e, Model of the anthranilate synthase assembly pathway.
Extended Data Fig. 4 | Cotranslational assembly of the phosphofructokinase complex. **a**, Domain organization of the phosphofructokinase (PFK) subunits. **b**, Engagement of nascent α and β subunits by C-terminally tagged α subunit (top) compared to engagement of nascent α and β by C-terminally tagged β subunit (bottom), analysed by SeRP. Data are from two biologically independent experiments. Coloured numbers indicate ribosome positions when the enrichment stably crosses the twofold threshold. The area between replicates is shaded, indicating the degree of experimental variation. **c**, Top, crystal structure of the *S. cerevisiae* PFK complex (PDB: 3O8O2). Bottom, crystal structure of the highly homologous (~75% sequence similarities) *Pichia pastoris* (also known as *Komagataella pastoris*) PFK complex, (PDB: 3OPY3). The N′-terminal glyoxalase I-like interface domains of α and β are outlined. This domain is missing in the *S. cerevisiae* structure, as the first 200 amino acids of each subunit, containing this domain were cleaved before crystallization. **d**, GFP tagging of the complex subunits does not affect cell growth with glucose as carbon source (YPD). A representative image from three biologically independent experiments is shown. **e**, Model of PFK assembly pathways.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Aggregation and degradation propensity of individual complex subunits. a, Stability of individual complex subunits, tagged by GFP, determined by CHX chase, in wild-type and deletion strains expressing orphan complex subunit. Cells with GFP fluorescence were analysed by FACS. Mean GFP fluorescence ± s.e.m. are presented with each data point from three biologically independent experiments overlaid. In each experiment, 20,000 events were recorded. **P = 0.0253, two tailed t-test. b, Solubility of individual complex subunits, tagged by GFP, determined by localization patterns changes, in wild-type and in deletion strains expressing orphan complex subunit. Log-phase cells (30 °C) were fixed and analysed by confocal microscopy (left). A representative image is shown. Scale bar, 4 μm. The fraction of cells displaying foci of GFP-tagged subunit per cell was quantified (right) (n = 155 cells per sample; from three biologically independent experiments). Data are mean ± s.e.m. overlaid with each data point. c, Subunit aggregation is complex-specific. Solubility of the Naa15–GFP subunit of the NatA complex in trp2Δ mutant cells deleted for the Trp2 subunit of the TRP complex, analysed as in b. (n = 155 cells/sup; from three biologically independent experiments). Data are mean ± s.e.m. overlaid with each data point. **P = 1.367248 × 10^{-11} (middle) and P = 7.850135 × 10^{-10} of a (lower panel) of a two tailed t-test. d, Characteristics of cotranslational complex assembly interactions. Left, zoom-in on the first 400 codons, displaying the onset and persistence of cotranslational interaction of each subunit with its partner subunit or subunits, for all 14 subunits identified as cotranslationally engaged. Right, the corresponding normalized length of each ORF at the onset of cotranslational interactions with partner subunits, demonstrating the length variability at the onset position.
Extended Data Fig. 6 | Proteome wide bioinformatics analysis of Ssb1 interplay with putative onset of cotranslational assembly interactions. 

**a**, Metagene analysis of Ssb1–GFP interaction profiles with the nascent chains of 116 yeast proteins identified as putative cotranslationally assembling subunits (putative assembly identification algorithm and parameters are detailed in the Supplementary Information). The dark grey line indicates Ssb interaction profiles, aligned to the subunits putative onset of cotranslational subunit association positions depicted as 0 (onset position alignment). A zoomed-in view of the nascent-chain segments at assembly onset position ±75 amino acids is shown. The orange line indicates Ssb binding profiles for nascent chains aligned to random positions along the ORFs. Data are from two biologically independent experiments. The area between replicates is shaded, indicating the degree of experimental variation. There is no correlation detected between the random and onset position alignment (Pearson correlation $r^2 = 0.2911$), thus Ssb depletion at positions of onset is significant.

**b**, Average Kyte–Doolittle hydrophobicity plot (7-amino-acid window) of the 116 nascent-chain segments. A zoomed-in view of the nascent-chain segments at assembly onset position ±75 amino acids is shown, as in **a**.
Extended Data Fig. 7 | Cotranslational interactions networks of FAS β, Cpa2 and PFK β metabolic enzymes subunits, analysed by SeRP.

a, Fatty acid synthesis metabolic pathway: nascent Faa1 is not engaged by C-terminally-tagged FAS complex β subunit, whereas nascent Acc1 shows a transient interaction, crossing the twofold enrichment threshold, at position approximately 250 codons/amino acids (indicated by an arrow).
b, Arginine biosynthetic pathway: nascent Arg4 (argininosuccinate lyase) is not engaged by C-terminally tagged Cpa2 subunit, whereas nascent Arg1 shows several transient interactions crossing the twofold enrichment threshold, at positions indicated by arrows. c, Glycolysis pathway: nascent Fba1 (fructose 1,6-bisphosphate aldolase) is not engaged by C-terminally tagged PFK complex β subunit, whereas Pyc2 (pyruvate carboxylase isoform) shows several transient interactions crossing the twofold enrichment threshold, at positions indicated by arrows. a–c, Data are from two biologically independent experiments. The area between replicates is shaded, indicating the degree of experimental variation.
Extended Data Fig. 8 | Model of cotranslational folding and assembly of complex subunits. a, Nascent chains emerging from the ribosome exit tunnel are first engaged by ribosome-associated chaperones. Upon emergence of a complete interaction domain the nascent chain is engaged by its complex partner subunit. This engagement remains stable throughout the rest of the ORF translation. b, The nascent-chain amino acid composition at the ribosome exit tunnel may direct the interplay between Ssb and partner-subunit association. High hydrophobicity and positively charged amino acids (aa) are engaged by Ssb; low hydrophobicity disfavours binding of Ssb at the onset of subunit association, allowing for folding of the interaction domain and subunit joining. c, Modes of cotranslational assembly: most complexes are assembled in a unidirectional manner, in which one dedicated, fully synthesized subunit engages its nascent partner. d, Diverging misfolding propensities of complex subunits: subunits engaged as nascent chains are prone to misfolding, whereas their partner subunits are generally more stable.
Extended Data Table 1 | Characteristics of the selected complexes in *S. cerevisiae*

| Complex                                           | Function                  | Nr. of Subunits*          |
|---------------------------------------------------|---------------------------|---------------------------|
| 1 Fatty Acid Synthase                             | Fatty acid synthesis      | 2 (α6β6)                  |
| 2 Aminoacyl-tRNA Synthetase complex                | Translation               | 3                         |
| 3 N-acetyltransferase A                           | Acetylation               | 2                         |
| 4 N-acetyltransferase B                           | Acetylation               | 2                         |
| 5 Anthranilate Synthase                           | Tryptophan biosynthesis   | 2                         |
| 6 Carbamoyl Phosphate Synthetase                  | Arginine biosynthesis     | 2                         |
| 7 Phosphofructokinase                             | Glycolysis                | 2 (α4β4)                  |
| 8 Translation Initiation Factor eIF2              | Translation               | 3 (α,β,γ)                 |
| 9 Nascent Chain Associated chaperone Complex (NAC)| Protein folding           | 2                         |
| 10 RiboNucleotide Reductase sub-complex RNR2,4    | dNTP synthesis            | 2                         |
| 11 V-type ATPase-Peripheral sub-complex,           | Vacuolar membrane ATPase complex | 2 (A₃,B₃)               |
| the catalytic core hexamer                         |                           |                           |
| 12 20S Proteasome sub-complex, α1,2 subunits      | Degradation               | 2 (α1-6, β1-6)            |

*Indicates the number of unique subunits analysed in the study. The total number of subunits in the complex, including repeating subunits, is shown in brackets.
Extended Data Table 2 | Characteristics of cotranslationally assembling subunits—directionality and aggregation propensities in ssb1/2Δ

| Complex                                      | Bait Subunit       | Nascent Polypeptide engaged | Aggregation propensity in Δssb1/2Δ |
|----------------------------------------------|--------------------|------------------------------|----------------------------------|
| 1 Fatty Acid Synthase                        | β                  | α                            | α, β                             |
| 2 Aminoacyl-tRNA Synthetase                  | GluRSp, Arc1p, MetRSp | GluRSp, Arc1p, MetRSp       | GluRSp, Arc1p, MetRSp            |
| 3 N-acetyltransferase A                      | Naa10              | Naa15                        | Naa10,15                         |
| 4 N-acetyltransferase B                      | Naa25              | Naa20                        | N.D                              |
| 5 Anthranilate Synthase                      | Trp2p              | Trp3p                        | Trp2p                            |
| 6 Carbamoyl Phosphate synthetase A           | Cpa2p              | Cpa1p, Cpa2p                 | N.D                              |
| 7 Phosphofructokinase                        | α, β               | α, β                         | α, β                             |
| 8 Translation Initiation Factor eIF2         | γ                  | β                            | γ, β                             |
| 9 Nascent chain Associated Complex           | α, β               | α, β                         | N.D                              |
| V-type ATPase-Peripheral sub-complex; Vma1,2 | N.D                | N.D                          | Vma1,2                           |
| 11 RiboNucleotide Reductase sub-complex      | N.D                | N.D                          | RNR2                             |
| 12 20S proteasome; α 1,2 subunits            | N.D                | N.D                          | α 1,2                            |

N.D, not detected.
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).

| n/a | Confirmed |
|-----|-----------|
| X   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| X   | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| X   | The statistical test(s) used AND whether they are one- or two-sided |
| X   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| X   | A description of all covariates tested |
| X   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| X   | 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) |
| X   | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted |
| X   | Give P values as exact values whenever suitable. |
| X   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| X   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| X   | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |
| X   | Clearly defined error bars |
| X   | 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 | MetaMorph Advanced Image Acquisition software, version 7.8.13.0, by Molecular devices LLC, was used for confocal imaging. |
| Data analysis  | cutadapt ver 1.8.3 https://pypi.org/project/cutadapt/ |
|               | Bowtie2 ver. 2.2.5.0 Langmead and Salzberg, 2012 http://bowtie-bio.sourceforge.net/bowtie2/index.shtml |
|               | Tophat2 ver. 2.0.13 Kim et al., 2013 http://ccb.jhu.edu/software/tophat/downloads/ |
|               | Python ver. 2.7 and ver. 3.4 Python Software Foundation https://www.python.org/downloads/ |
|               | ImageJ software, ver 1.50i. https://imagej.nih.gov/ij/ |

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

The selective ribosomal profiling (SeRP) raw data sets relating to figures: 1-4 and extended data figures 1,4,5 and 7 are publicly available, upon publication, accession number GEO: GSE93830. Figure 4 and extended data figure 6 rely also on raw data derived from the publicly available data sets of Ssb1 SeRP experiments, accession number GEO: GSE93830.

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 | No sample size calculation was performed, as our analysis is based on the parallel measurements of billions of yeast cells per experiment (~5.6X10^9 cells, according to O.D measurements at 600nm). This sample size is by definition very high. |
| Data exclusions | No data were excluded from the analyses. Minimal detection thresholds were used for including each gene in the analysis (A threshold of 64 total counts per gene was chosen as a point where the inter-replicate variation approached its infinite-counts asymptote and counting statistics contributed little. As in: Ingolia, N.T. et al., Science. 10; 324(5924)(2009)). |
| Replication | We used a minimal of 2 independent biological replicates per experiment. All replication attempts were successful. For each specific experiment the number of replicates is indicated in the text or figure legend. The replicates were highly reproducible as indicated by the shaded area between SeRP replicates, indicating the degree of experimental variation. Similarly, imaging stability assays and qPCR results all showed high reproducibility. |
| Randomization | Only relevant for Metagene analysis, Figure 4. The randomization code: (based on- https://stackoverflow.com/questions/3996904/generate-random-integers-between-0-and-9) from random import randint print(randint(A, B)) A = 30 (30 amino acids from the N terminus are ignored because of the tunnel) B = End of the gene or gene length |
| Blinding | Not relevant for this study, as it does not include animals and/or human research participants. Furthermore, data analysis was performed mainly bioinformatically, using dedicated scripts. |

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

All unique materials used in this study are readily available from the authors or from standard commercial sources, as described in the Methods for each specific experiment.

Antibodies

Antibodies used

Polyclonal rabbit FAS antibody, S. cerevisiae as described in: Egner, R. et al. JBC 268, 27269-27276 (1993), [a gift from Dieter H. Wolf, Stuttgart University, Stuttgart, Germany], 1:5000 dilution.
Polyclonal rabbit GFP antibody (antiserum from rabbit raised against YFP) (Haslberger, T. NSMB 15(6):641-50 (2008). 1:5000 dilution.

Validation

FAS antibody, as described in: Egner, R. et al. JBC 268, 27269-27276 (1993).
GFP antibody, as described in: antiserum from rabbit raised against YFP (Haslberger, T. NSMB 15(6):641-50 (2008).
Proteins were visualized by enhanced chemi-fluorescence reaction, each data sets contains positive results, as described in the manuscript.

Flow Cytometry

Plots

Confirm that:

☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☑ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☑ All plots are contour plots with outliers or pseudocolor plots.
☑ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Yeast cells were grown to log phase, then cycloheximide (0.5 mg/ml) was added, and aliquots from each time point were taken. GFP levels of fixed cells at each time point were determined by Flow Cytometry analysis performed using FACS.

Instrument

BD FACS Canto II equipped with Lasers 405nm, 488 nm, 635nm.

Software

BD FACSDiva 8.0.1

Cell population abundance

Cell population abundance was determined by using the following detectors: FSC, SSC, 488-E for GFP with filter 530/30.

Gating strategy

Cell population gated on FSC/SSC area dot plot, exclusion of debris and cell aggregates additionally by SSC/FSC height and width.

☑ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.