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Reversible amyloids of pyruvate kinase couple cell metabolism and stress granule disassembly

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Abstract

Cells respond to stress by blocking translation, rewiring metabolism, and forming transient mRNP assemblies called stress granules (SGs). After stress release, re-establishing homeostasis requires energy-consuming processes. However, the molecular mechanisms whereby cells restore energy production to disassemble SGs and reinitiate growth after stress remain poorly understood. Here we show that, upon stress, the ATP-producing enzyme Cdc19 forms inactive amyloids, and that their rapid re-solubilization is essential to restore energy production and disassemble SGs. Cdc19 re-solubilization is initiated by the glycolytic metabolite fructose-1,6-bisphosphate (FBP), which directly binds Cdc19 amyloids and facilitates conformational changes that allow Hsp104 and Ssa2 chaperone recruitment. FBP then promotes Cdc19 tetramerization, which boosts its activity to further enhance ATP production and SG disassembly. Together, these results describe a molecular mechanism essential for stress recovery, which directly couples metabolism with SG dynamics *via* regulation of Cdc19 amyloids.

Introduction

Cells respond to diverse intra- and extracellular signals as well as environmental changes, and adjust their activities to maintain homeostasis. In particular, cells need to rapidly respond to potentially harmful stress conditions such as osmolarity changes, oxidative or heat stress and nutrient starvation [1]. To cope with these challenges, cells developed complex mechanisms to sense various stress conditions and orchestrate appropriate protective responses, including inhibition of cell growth and cell cycle progression, activation of specific transcriptional programs, and formation of mRNP assemblies known as stress granules (SGs) [1, 2]. SGs are dynamic cytoplasmic membrane-less organelles that transiently form in response to metabolic stress and are rapidly disassembled under favourable growth conditions. They contain several RNA-binding proteins and metabolic enzymes [3], and are thought to play an important role in maintaining homeostasis by sequestering cellular components and protecting them from stress-induced degradation, and/or by concentrating them to favour specific interactions [4-6]. Alterations in SG dynamics and the consequent metabolic changes have been implicated in the pathogenesis of several human neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and frontotemporal lateral dementia (FTD) [7, 8]. Indeed, chronic stress or mutations leading to persistent SGs have been shown to facilitate the formation of irreversible, pathological protein aggregates, suggested to promote aging and aging-associated diseases [8]. SGs thus need to be carefully controlled and efficiently disassembled. However, despite their pathophysiological importance, the mechanisms regulating SG dynamics remain poorly understood.

Most SG proteins contain intrinsically disordered low-complexity regions (LCRs) that are able to condense by liquid-liquid phase separation (LLPS) [9, 10]. Together with low-affinity RNA-RNA interactions, this LLPS process is thought to underlie, at least in part, the formation of SGs [11, 12]. Several factors are known to influence LLPS, including post-translational modifications, energy availability, temperature, and biophysical properties of the cytoplasm, such as crowding and viscosity [13-17]. In contrast, the mechanisms regulating SG disassembly are less well understood, but ATP seems to play a crucial role in this process. Indeed, artificial ATP depletion reduces SG dynamics and results in persistent SGs [3, 9, 18]. In contrast, adding ATP to stressed cells is sufficient to trigger

aggregate disassembly [19]. Thus, ATP availability is essential and sufficient to initiate SG disassembly, and energy-consuming activities including autophagy, as well as ATP-dependent enzymes such as chaperones and RNA/DNA helicases have been implicated in this process [20-26]. For instance in yeast, the disaggregase Hsp104 and the Hsp70 chaperones Ssa1 and Ssa2 accumulate in SGs, and their ATP-dependent activities are important for cells to efficiently disassemble SGs and restart growth after stress release [19, 20]. The ATP-dependent kinases Sky1 [22] and ULK1/2 [23] have also been implied in efficient SG disassembly, and ATP itself has been proposed to act as a biological hydrotrope to modulate cytoplasmic diffusion and further facilitate aggregate re-solubilization [27]. Intriguingly, however, ATP reserves are rapidly depleted in stressed cells [19, 28-31], raising the question of how energy production is restored after stress release to allow efficient SG disassembly.

Cells mainly produce ATP by fermenting glucose through glycolysis [32, 33]. In this pathway, the ATP-producing and irreversible final step is carried out by the essential and evolutionarily conserved enzyme pyruvate kinase (Cdc19 in yeast), which catalyses the conversion of phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP. Cdc19 activity is influenced by its allosteric regulator, fructose-1,6-biphosphate (FBP), whose binding promotes Cdc19 oligomerization into more active tetramers [4, 31, 34, 35]. Moreover, flux through glycolysis is modulated by trehalose metabolism, which regulates glucose-6-phosphate availability and hexokinase activity [36-38]. Interestingly, we previously showed that in response to glucose starvation and heat stress, Cdc19 reversibly aggregates *via* its aggregation-prone LCR and localizes to SGs [4, 39]. Formation and disassembly of Cdc19 aggregates modulate SG dynamics, and play a crucial role in stress survival. Indeed, aggregating Cdc19 protects this essential enzyme from stress-induced degradation, and accelerated Cdc19 aggregation leads to faster SG formation. In contrast, re-solubilization of Cdc19 aggregates is required to dissolve SGs and re-activate TORC1 and PKA growth pathways after stress release [4]. Mutations in the Cdc19 LCR that cause irreversible aggregates are also shown to compromise SG disassembly and cell cycle restart after stress. Cdc19 is remarkable in that it forms stable amyloid-like aggregates *in vitro*, while *in vivo* these aggregates are fully reversible within minutes after stress release. As amyloids are characterized by high resistance to enzymatic degradation,

this conformation would be well suited to avoid degradation during stress *in vivo*, but would raise the question of how these structures can be readily disassembled once stress is released. However, the structural nature of physiological Cdc19 aggregates, and the molecular mechanisms governing their disassembly *in vivo* remain unclear. Likewise, the physiological relevance of reversible amyloid-like Cdc19 aggregates in SG regulation and growth restart after stress is poorly understood.

To address these questions, we exploited a mutant of Cdc19 (Cdc19^{irrev}) which is unable to disassemble Cdc19 aggregates after stress [4], and combined *in vitro* and *in vivo* approaches to elucidate the molecular mechanisms responsible for Cdc19 re-solubilization. Moreover, we further characterized the structure of reversible Cdc19 aggregates *in vivo*, showing that they assume an amyloid structure also within cells. Interestingly, we found that formation of Cdc19 amyloids inactivates the enzyme, thus abolishing ATP production during stress. Re-solubilization of these aggregates after stress release is necessary to restore ATP production and re-solubilize SGs, and we propose a molecular mechanism describing how functional Cdc19 amyloids are disassembled. In particular, we demonstrate that direct binding of FBP to Cdc19 amyloids initiates disassembly by promoting a conformational change that is required to trigger recruitment of the molecular chaperones Hsp104 and Ssa2, and to allow Cdc19 amyloid re-solubilization and subsequent SG disassembly. Together, our studies not only provide a mechanism for re-solubilizing functional amyloids *in vivo*, but also show how metabolism and energy production are coupled to SG dynamics and cell growth by regulating both assembly and disassembly of reversible, functional Cdc19 amyloids.

Results

Re-solubilization of Cdc19 amyloids is required to restore ATP production and disassemble SGs

Formation of reversible Cdc19 aggregates upon stress is required to ensure cell survival [4]. We found that upon return to favourable growth conditions, re-solubilization of Cdc19 aggregates is essential to both disassemble SGs and re-initiate growth [4]. Indeed, cells expressing a functional but irreversibly aggregating mutant of Cdc19 (Cdc19^{irrev}, Suppl. Fig 1A) are unable to survive stress and

cannot disassemble SGs (Fig. 1A). Interestingly, Cdc19^{irrev} aggregates not only fail to re-solubilize after stress, but also cause persistent SGs (Fig. 1A). In order to understand how Cdc19 regulates SG disassembly and the mechanisms underlying Cdc19 aggregate re-solubilization, we investigated wild-type and Cdc19^{irrev} aggregate properties and potential differences *in vitro* and *in vivo*.

First, we examined biophysical properties of wild-type and Cdc19^{irrev} aggregates, to understand if irreversibility was caused by an aberrant aggregate structure. To this end, wild-type and mutant proteins were expressed and purified from *E. coli*, and their aggregation was triggered *in vitro* with a brief heat shock treatment. Upon heat shock, both proteins formed needle-like filaments, which were indistinguishable by negative staining transmission electron microscopy (TEM) analysis (Fig. 1B). Both wild-type and mutant aggregates also revealed similar β -sheet rich-structures, as shown by circular dichroism (CD) measurements (Fig. 1C), and could bind the amyloid dyes Thioflavin T (ThT) and Congo Red (CR) [40] (Suppl. Fig. 1B and C), suggesting that wild-type and Cdc19^{irrev} proteins form similar amyloids *in vitro*. To test whether Cdc19 aggregates exhibit amyloid structures *in vivo*, we subjected yeast cells to heat shock, lysed them, and stained both wild-type and Cdc19^{irrev} aggregates with CR. As shown in Suppl. Figure 1D, cellular Cdc19 aggregates could be stained with the amyloid dye in both cases, while no Cdc19 aggregates were visible in control conditions. Background CR staining is likely due to the ability of CR to bind cellular structures (e.g. cell wall) other than amyloids [41]. To further confirm the amyloid nature of Cdc19 aggregates *in vivo*, and to better compare the structure of wild-type and Cdc19^{irrev} aggregates, we applied limited proteolysis coupled to mass-spectrometry (LiP-MS). This method allows to probe subtle conformational changes both *in vitro* and in complex cell lysates, and offers more precise structural information compared to conventional methods for detecting amyloids [42, 43]. Thus, we used LiP-MS to compare the structural alterations undergone by wild-type and Cdc19^{irrev} upon aggregation *in vivo* and *in vitro* (Suppl. Fig. 1E). Cdc19 aggregation was induced *in vitro* by heat shocking purified Cdc19, and *in vivo* by subjecting cells to starvation stress to achieve full amyloid formation. This analysis revealed that diagnostic Cdc19 LiP-MS peptides characteristic of the amyloid conformation *in vitro* resemble the structures observed *in vivo*, thus corroborating the notion that Cdc19 aggregates are amyloids both *in vitro* and *in vivo*.

Moreover, conformation-sensitive LiP-peptides were mostly similar in wild-type and Cdc19^{irrev} assemblies, suggesting that the different re-solubilization behaviour of these proteins is not due to the formation of two radically different structures (Suppl. Fig. 1E). However, LiP-MS identified subtle structural alterations in the aggregated catalytic domain of Cdc19. We therefore asked whether Cdc19 activity is affected by its aggregation and re-solubilization, and examined potential differences between Cdc19^{WT} and Cdc19^{irrev}.

First, we compared the enzymatic activity of soluble and aggregated Cdc19 using an established lactate-dehydrogenase-coupled pyruvate kinase activity assay. Cdc19 amyloids were enzymatically inactive (Fig. 1D), suggesting that the active site in these reversible assemblies may be inaccessible to convert PEP into ATP. Given the large conformational changes undergone by the protein to form amyloid fibrils, the loss of catalytic activity is not surprising. This is also in accordance with previous observations showing that in cells exposed to heat stress or glucose starvation (i.e. when Cdc19 is aggregated) ATP levels rapidly drop, while conversely Cdc19's substrate PEP accumulates under these conditions [19, 29-31] (Fig. 1F). To re-activate Cdc19 and resume ATP production, Cdc19 amyloids thus need to be re-solubilized. Since high ATP levels are essential to disassemble SGs, we asked whether persistent SGs in *cdc19^{irrev}* cells could be due to impaired ATP production. Indeed, we found that while wild-type cells presented elevated ATP levels and low PEP during stress recovery, *cdc19^{irrev}* cells could not metabolize the accumulated PEP and hence failed to restore ATP production (Fig. 1E). We conclude that wild-type and *cdc19^{irrev}* cells differ primarily in their ability to restore energy production after stress.

To test if increasing ATP levels was sufficient to disassemble SGs, we restored energy metabolism in heat stressed *cdc19^{irrev}* cells by two different Cdc19-independent means. First, we artificially overexpressed Pyk2, a pyruvate-kinase isoform that is expressed at very low levels in glucose-rich conditions but like Cdc19 uses PEP to produce ATP [44]. In accordance with our hypothesis, restoring ATP production by overexpressing Pyk2 re-solubilized Cdc19^{irrev} aggregates and SGs, and restored growth of *cdc19^{irrev}* cells exposed to heat stress (Fig. 1G and H). Second, we grew *cdc19^{irrev}* cells with 2% ethanol instead of glucose. Under these conditions, ATP production occurs

mainly *via* oxidative phosphorylation independently of Cdc19 [45]. Also in this case, having an alternative ATP source allowed re-solubilization of Cdc19^{irrev} aggregates and SGs (Fig. 1I). We conclude that under physiological conditions, re-solubilizing Cdc19 amyloids is critical to resume ATP production after stress, which in turn is likely required to power ATP-dependent processes to dissolve SGs and restart cell growth.

Re-solubilization of Cdc19 amyloids *in vivo* is triggered by its allosteric regulator fructose-1,6-bisphosphate (FBP)

To better understand the molecular mechanisms regulating Cdc19 amyloid disassembly, we performed a genetic suppressor screen to identify mutants that are able to restore growth of stressed *cdc19^{irrev}* cells (Suppl. Fig. 2A). Briefly, we subjected exponentially growing cells expressing GFP-tagged Cdc19^{irrev} to a 42 °C heat shock treatment for 30 min, and plated the cells at 30 °C to isolate rare spontaneous suppressors which were able to resume growth after stress. We deleted *pyk2* in the isolated mutants to exclude the possibility that suppression is mediated by upregulating Pyk2 activity, and then backcrossed to wild-type cells to confirm Mendelian segregation of the phenotype. Using whole genome sequencing, we identified a single point mutation in *Tps3*, a protein involved in trehalose biosynthesis, as the likely cause for the Cdc19^{irrev} suppressor phenotype (Suppl. Fig. 2B and C). Trehalose is a disaccharide which is synthesized in a two-steps process (Fig. 2A). In a first step, *Tps1* catalyses the conversion of glucose-6-phosphate (G6P) into trehalose-6-phosphate (T6P), and *Tps2* then converts T6P into trehalose. *Tps3* acts as cofactor, facilitating this trehalose production. Deletion of either *tps1*, *tps2*, or *tps3*, which abrogates trehalose production (Suppl. Fig. 2E, [46]), did not alter growth compared to wild-type controls (Suppl. Fig. 2D), but allowed *cdc19^{irrev}* cells to overcome stress-induced growth arrest and partially restored growth after stress release (Fig. 2B), suggesting that decreasing trehalose metabolism favours Cdc19 re-solubilization. As *Tps1* catalyses the first reaction in the trehalose synthesis pathway and its deletion resulted in the strongest rescue (Fig. 2B), we first focussed on this mutant. Fluorescence microscopy confirmed that growth restart correlated with re-solubilization of Cdc19^{irrev} amyloids and SG disassembly in the *tps1Δ* strain (Fig. 2C). As expected, Cdc19 re-solubilization in turn coincided with restored ATP production after stress release. Indeed,

metabolomic data showed that *cdc19^{irrev}* cells fail to produce high ATP concentrations and instead maintain high intracellular PEP levels after stress release, while *cdc19^{irrev} tps1Δ* cells consume PEP and can resume ATP production (Fig. 2D).

We next wanted to understand how Tps1 deletion allows *cdc19^{irrev}* amyloid disassembly. We reasoned that Tps1 activity decreases glycolytic flux on the one hand by diverting G6P from glycolysis into trehalose synthesis, and on the other hand by generating T6P, which in turn inhibits hexokinase (Fig. 2A, [36-38]). As a result, *tps1Δ* cells contain virtually no trehalose, but have enhanced flux through glycolysis, which results in increased levels of the glycolytic intermediate fructose-1,6-bisphosphate (FBP) [38]. FBP levels tightly correlate with glycolytic flux and cell growth, with low FBP reflecting low glycolysis and a stressed, quiescent cell state, while high FBP levels denote high glycolytic flux and metabolic conditions favourable for growth [47, 48]. Interestingly, besides being a “glycolysis flux sensor”, FBP is also a known allosteric regulator of Cdc19 [31]. We thus hypothesized that increased FBP levels in *tps1Δ* cells may directly or indirectly play a role in the disassembly of Cdc19 aggregates. To test this, we compared intracellular trehalose and FBP levels in *cdc19^{irrev}* and *cdc19^{irrev} tps1Δ* cells exposed to heat shock conditions. As expected, deletion of Tps1 abrogated the production of the disaccharide trehalose (Fig. 2E). Moreover, similar to glucose starvation [38], heat stress induced a rapid decrease in FBP, but during stress recovery this metabolite then accumulated to significantly higher levels in *cdc19^{irrev} tps1Δ* mutants compared to *cdc19^{irrev}* controls (Fig. 2F). To genetically test whether the observed rescue of stressed Cdc19^{irrev} mutants by *tps1Δ* is mediated by increased FBP levels, we deleted Pfk2, the main FBP-producing enzyme in glycolysis, to impair FBP production [38]. Interestingly, in contrast to *tps1Δ*, *tps1Δ pfk2Δ* double mutants were unable to recover from heat stress, while *pfk2Δ* alone neither significantly reduced growth rates nor impaired survival to stress (Fig. 2G), despite comparable expression levels of the Cdc19 variants (Suppl. Fig 2F). Moreover, *tps1Δ pfk2Δ* cells were unable to dissolve Cdc19^{irrev} aggregates and SGs (Fig. 2H). Thus, we conclude that high FBP levels contribute to efficient stress recovery and Cdc19 amyloid and SG disassembly.

Altered trehalose levels might also influence Cdc19 resolubilization by other means, as trehalose has been shown to affect SG dynamics by tuning cytoplasmic viscosity [15]. Indeed, also deleting Tps2, the enzyme catalysing the last step in the trehalose synthesis pathway, weakly rescues the stress recovery and aggregate dissolution defects observed in *cdc19^{irrev}* cells (Suppl. Fig. 2G), although this effect is much weaker compared to *tps1Δ* cells (Fig. 2B). While both strains are impaired in trehalose synthesis, only deletion of Tps1, and not Tps2, resulted in increased FBP levels after stress in *cdc19^{irrev}* cells (Suppl. Fig. 2H). We thus conclude that FBP-dependent and –independent mechanisms might co-operate to achieve efficient Cdc19 amyloid disassembly upon stress release, although increasing cytoplasmic viscosity *via* trehalose breakdown plays a minor role compared to increased FBP levels.

FBP directly regulates the formation and disassembly of reversible Cdc19 amyloids

Our results suggest that FBP serves as a sensor to couple cellular metabolism and energy production with SG disassembly and cell growth, by directly modulating Cdc19 aggregation and catalytic activity (Fig. 2). Two mechanisms could be envisioned to explain how direct FBP binding could regulate this process (Fig. 3A). First, FBP could reduce aggregation by promoting Cdc19 tetramerization (Fig. 3A, arrow 1), as Cdc19 tetramers are less aggregation-prone than monomers [4]. Alternatively, FBP could directly bind to Cdc19 amyloids and induce conformational changes that lead to Cdc19 amyloid and SG disassembly (Fig. 3A, arrow 2). These two modes of action are not mutually exclusive, and could co-operate to ensure rapid Cdc19 re-solubilization. To elucidate the mechanism underlying FBP-mediated regulation of Cdc19 amyloids, we next investigated the effects of FBP on the formation and disassembly of Cdc19 aggregates. First, we biochemically compared Cdc19 amyloid formation in the presence or absence of FBP and other metabolites *in vitro* (Fig. 3B-D). Incubation of purified Cdc19^{WT} with 5 mM FBP, but not its structurally-related precursor fructose-6-phosphate (F6P), interfered with aggregate formation as demonstrated by a simple centrifugation assay (Fig. 3B). Indeed, in the presence of FBP, a significant portion of Cdc19^{WT} remained soluble, with a corresponding decrease in the aggregate pellet fraction. Conversely, addition of 5 mM F6P, ATP, PEP or trehalose did not influence Cdc19 aggregation (Suppl. Fig. 3A-C). To confirm that FBP reduces Cdc19 aggregation

by directly binding to the protein, we next measured the aggregation behaviour of a Cdc19 point-mutant (T403E) unable to bind FBP (Cdc19^{ΔFBP}, [49]). As shown in Figure 3C, addition of FBP did not interfere with heat shock-induced pelleting of Cdc19^{ΔFBP}. Taken together, these data show that direct binding of FBP to Cdc19 monomers prevents amyloid formation in part by promoting the formation of non-aggregating Cdc19 tetramers (Fig. 3A, arrow 1). However, FBP did not affect the formation of Cdc19^{irrev} aggregates (Fig. 3D), suggesting that this mutant might have a reduced FBP-binding ability and/or be unable to tetramerize. Indeed, size-exclusion chromatography demonstrated that the Cdc19^{irrev} protein is fully monomeric both *in vivo* and *in vitro* (Fig. 3E and F). Importantly, these results imply that the FBP-mediated rescue of the Cdc19^{irrev} mutant *in vivo* cannot simply be explained by increased tetramerization (Fig. 3A, arrow 1), but rather FBP must also promote aggregate disassembly (Fig. 3A, arrow 2). To confirm that direct binding of FBP to Cdc19 amyloids is required for their disassembly, we compared aggregation dynamics of wild-type Cdc19 and the FBP binding-deficient Cdc19^{ΔFBP} mutant *in vivo* (Fig. 3G). Interestingly, while the Cdc19^{ΔFBP} mutant protein was soluble under favourable growth conditions and rapidly aggregated upon heat shock, Cdc19^{ΔFBP} aggregates were irreversible and unable to disassemble after stress release. In contrast to Cdc19^{irrev} aggregates, Cdc19^{ΔFBP} aggregates could not be rescued by increasing FBP levels by *TPS1* deletion. Taken together, these data show that direct binding of FBP to Cdc19 is essential to disassemble Cdc19 amyloids *in vivo*.

Next, we examined whether FBP is sufficient to disassemble pre-formed Cdc19 amyloids *in vitro*. We induced fibril formation of purified wild-type Cdc19 by heat shock, and incubated the obtained amyloids with varying concentrations of FBP. After several hours, the aggregates were pelleted by centrifugation and the soluble and insoluble fractions analysed by SDS-PAGE. As shown in Suppl. Fig. 3 D, we did not observe significant re-solubilisation of Cdc19 even upon incubation with excess FBP (20 mM), indicating that FBP binding is not sufficient to disassemble Cdc19 amyloids *in vitro*. However, we found that FBP is able to alter the morphology of Cdc19 amyloids. Indeed, incubation of pre-formed Cdc19^{WT} aggregates with FBP resulted in clear morphological alterations as shown by negative staining TEM (Fig. 3H, +FBP). Formerly straight fibrils developed protruding globular bulges and several regions of mild curvature along the fibril axis. Furthermore, larger globular

assemblies appeared close to the fibrils, and the fibrils were consistently positively stained (i.e. of dark colour). This change from negative (white fibrils) to positive (black fibrils) staining results from acidic FBP molecules bound to the fibrils that attract significantly more of the positively charged uranyl stain. In contrast, incubation of pre-formed Cdc19^{WT} amyloids with buffer (Fig. 3H, buffer) or the FBP “analogue” F6P (Fig. 3H, +F6P) did not cause any morphological changes and the Cdc19^{WT} amyloids appeared as needle-like fibrils characterized by smooth edges, thus confirming FBP-specificity. As expected, Cdc19^{ΔFBP} amyloid fibrils incubated with FBP also retained their straight, smooth morphology, confirming that the observed conformational changes are induced by direct FBP binding to Cdc19 (Fig. 3H, lower panels). Together, these striking differences suggest that FBP directly binds to Cdc19 amyloids and induces pronounced conformational changes along the fibril. While these alterations are not sufficient to trigger Cdc19 amyloid re-solubilization *in vitro*, they may facilitate binding of other factors that together promote the efficient amyloid disassembly observed *in vivo*.

Chaperones co-operate with FBP to achieve efficient re-solubilization of Cdc19 amyloids *in vivo*

Untangling of cellular misfolded protein aggregates is mediated by different chaperones, including Hsp104 and members of the Hsp70 (Ssa1-4) and the Hsp110 families (Sse1-2) [20, 50, 51]. Certain chaperones, such as Hsp104 and Ssa2, have also been implicated in re-modelling functional protein assemblies and SGs, and indeed cells lacking *HSP104* or *SSA1/2* are slightly delayed in SG disassembly [50]. We thus asked whether these chaperones could also be involved in facilitating disaggregation of Cdc19 amyloids. Interestingly, we observed that inhibiting Hsp104 activity using Gdn-HCl caused a delay in dissolution of both wild-type Cdc19 aggregates, as well as Cdc19^{irrev} aggregates in *tps1*Δ cells (Fig. 4A and B). Conversely, overexpressing Hsp104 was sufficient to at least partially restore growth of heat stressed *cdc19^{irrev}* cells (Fig. 4C), and this effect was further enhanced by simultaneously co-overexpressing Ssa2 (Suppl. Fig. 4A and B). Moreover, Hsp104 overexpression slightly improved growth of *cdc19^{irrev} tps1*Δ mutants after heat stress, indicating that increased FBP and chaperone levels have an additive effect (Fig. 4D). Increasing FBP levels by *TPS1* deletion allowed cells to rapidly restart growth after stress release, while Hsp104 overexpression seemed to take effect in facilitating growth at a later phase, suggesting that high FBP may be particularly important in the

initial steps of Cdc19 amyloid disassembly (Suppl. Fig. 4C). Together, these results imply that FBP binding to Cdc19 initiates aggregate re-solubilization and co-operates with Hsp104 and Ssa2 to disassemble SG *in vivo*. To further investigate the interplay between FBP levels and chaperones in the disassembly of Cdc19 amyloids, we compared the localization of Cdc19-GFP and Hsp104-mCherry or Ssa2-mCherry in wild-type, *cdc19^{irrev}* and *cdc19^{irrev} tps1Δ* cells. As expected, fluorescence microscopy confirmed that Hsp104-mCherry and Ssa2-mCherry co-localized with heat shock-induced Cdc19-GFP aggregates in the majority of wild-type cells (Fig. 4E and F). Surprisingly, however, Hsp104 and Ssa2 were not recruited to irreversible Cdc19^{irrev} aggregates but rather remained dispersed throughout the cytoplasm. Co-localization was restored upon deletion of *tps1*, consistent with the notion that increased FBP levels and FBP binding to Cdc19 allow chaperone recruitment to SGs (Fig. 4E and F) to promote Cdc19 and SG re-solubilization (Suppl. Fig. 4D). To corroborate these results, we next examined co-localization of Hsp104-mCherry with GFP-tagged Cdc19^{AFBP}. Indeed, Cdc19^{AFBP} amyloids failed to recruit Hsp104-mCherry, thus confirming that FBP binding to Cdc19 is essential for chaperone recruitment (Fig. 4G). In this background Hsp104-mCherry showed a punctate pattern by binding to aggregates formed upon heat stress, but these foci did not co-localize with irreversible Cdc19^{AFBP} amyloids (Fig. 4G). As expected, co-localization with Cdc19^{AFBP} aggregates was not restored in *tps1Δ* cells. We thus conclude that FBP-binding to Cdc19 aggregates triggers a conformational change that promotes chaperone binding and allows their recruitment to SGs, which in turn is essential for Cdc19 amyloid re-solubilization, resumption of ATP production, and SG disassembly.

Discussion

Cell survival under stress conditions requires rapid and reversible implementation of protective measures, including reprogramming of cellular metabolism, cytoplasmic re-organization and activation of selective autophagy [1]. A key step in this process is the formation of stress granules (SGs), highly dynamic, cytoplasmic, membrane-less organelles that are thought to store and protect mRNAs as well as proteins from stress-induced degradation [3]. Formation and disassembly of SGs needs to be tightly

regulated, as persistent SGs have been associated with several human pathologies [3]. Among the proteins regulating SG dynamics is the ATP-producing enzyme Cdc19. Cdc19 assembles into solid but reversible aggregates and localizes to SGs upon stress [4]. Once cells are released from stress, re-solubilization of Cdc19 aggregates is an essential step in disassembling SGs and restoring cell growth. However, the mechanisms regulating Cdc19 aggregate re-solubilization and their role in SG disassembly was poorly understood. Here, we show that reactivating energy metabolism is essential for SG disassembly, and propose a mechanism that links cellular metabolism and energy production to the assembly state of Cdc19.

Cdc19 forms catalytically inactive, reversible amyloids in response to stress conditions

Untimely degradation of essential proteins such as Cdc19 has lethal consequences. During stress, proteosomal degradation is upregulated [52] and misfolded proteins are often degraded through various pathways [53]. Therefore, essential proteins required for restarting growth need to be protected from degradation [4]. Several lines of evidence suggest that stress-induced Cdc19 aggregates assemble into a proteasome-resistant amyloid structure, similar to irreversible pathological aggregates characteristic of neurodegenerative diseases [39], while preserving the capacity to resolubilize after returning to stress-free conditions. Indeed, our CD measurements, amyloid-specific dye stainings, and TEM analyses demonstrate that purified Cdc19 forms amyloids *in vitro*. Furthermore, *in vivo* FRAP measurements [4] and staining of Cdc19 aggregates isolated from permeabilized yeast cells with the amyloid-specific dye Congo Red indicate that Cdc19 aggregates acquire solid, amyloid structures also *in vivo*. Finally, structural analysis by LiP-MS confirmed that Cdc19 aggregates *in vivo* resemble the amyloids formed by purified proteins *in vitro*. Taken together, we conclude that Cdc19 aggregates avoid stress-induced degradation *in vivo* owing to their proteolysis-resistant amyloid structure. These rigid assemblies may also have a scaffolding function and influence the structural integrity and dynamics of SGs. The comparison of Cdc19^{WT} and Cdc19^{irrev} aggregates further highlights that the structural features of physiological, reversible amyloids and irreversible structures are remarkably similar. It is worth mentioning that even single amino acid changes can be sufficient to transform reversible aggregates into aberrant entities associated with diseases, even without major structural changes [54].

Interestingly, we found that aggregation not only affects Cdc19 degradation, but also impairs its activity and hence ATP production. The formation of catalytically inactive Cdc19 amyloids results in a rapid decrease of cellular ATP levels and accumulation of glycolytic intermediates such as PEP during stress [31]. Thus, stress-induced Cdc19 aggregates are distinct from glycolytic bodies, which are thought to increase glycolytic flux in response to hypoxic stress by coalescing glycolytic enzymes [55]. Therefore, while in some cases concentrating molecular interactions or fixing favourable conformations promotes enzymatic reactions, the stress-induced structural rearrangements of Cdc19 prevent catalytic activity. While LiP-MS measurements exposed small conformational changes in the catalytic site, detailed structural information is required to understand how catalysis is inhibited in Cdc19 amyloids. Nevertheless, it is likely that the catalytic site undergoes a conformational change and/or becomes inaccessible to substrate when Cdc19 monomers assemble into higher order structures upon stress. In summary, forming solid amyloids protects Cdc19 from degradation and inhibits glycolysis during stress, while preserving the rapid re-initiation capability of cell growth.

FBP binding regulates disassembly of Cdc19 amyloids by recruiting molecular chaperones

Our data show that signals from glycolysis and cell cycle converge onto Cdc19, which in turn controls SG dynamics and cell survival during stress. Previous experiments indicate that FBP levels tightly correlate with the metabolic state of the cell, with low FBP levels characterizing stressed or quiescent cells, and high FBP levels representing metabolic conditions favourable for growth [47, 48]. FBP levels also signal stress-induced changes in glycolysis, implying that FBP functions as a “metabolic sensor” for glycolytic flux.

Our results suggest that FBP regulates the assembly state and activity of Cdc19 by two mechanisms (Fig. 5). First, during exponential growth, FBP binds to soluble Cdc19, promoting its tetramerization. In tetramers, the aggregation-prone LCR of Cdc19 forms an α -helix buried at the subunit interface, and is thus inaccessible to seed amyloids [4]. In contrast, low FBP levels characteristic of stressed conditions allow the formation of Cdc19 monomers, and the exposed LCR can trigger aggregation. Second, upon stress release, glycolysis restarts and FBP levels reach a critical concentration that facilitates Cdc19 re-solubilization by directly binding Cdc19 amyloids, inducing

conformational changes needed to trigger their disassembly. Indeed, cells expressing Cdc19^{AFBP} mutant protein, which cannot bind FBP, form aggregates that are unable to disassemble after stress release. Genetically increasing FBP levels by deleting *TPS1* could not rescue this phenotype, but was sufficient to allow disassembly of amyloids formed by the otherwise irreversible Cdc19^{irrev} mutant. Since the Cdc19^{irrev} protein is exclusively monomeric *in vitro* and *in vivo*, it is likely that it exhibits decreased FBP binding affinity, which can be overcome by increased FBP levels. Although FBP is not sufficient to disassemble amyloid Cdc19 fibrils *in vitro*, TEM analysis showed that FBP binding directly triggers profound morphological alterations along the entire fibrils axis that may help to dissolve these rigid structures. Based on these data we conclude that FBP links cellular metabolism to Cdc19 activity and regulates both the formation and disassembly of Cdc19 amyloids *in vivo*.

The dissolution of aggregated proteins commonly requires the help of molecular chaperones. In yeast, Hsp104 and Ssa1/Ssa2 play important roles in both counteracting and clearing aberrant protein aggregates [20, 51], as well as in regulating the formation and disassembly of functional structures such as SGs. Interestingly, we found that following FBP binding to Cdc19 aggregates, Hsp104 and Ssa2 are required for re-solubilization. Both Hsp104 and Ssa2 co-localize with Cdc19 amyloids in SGs, and their ATP-dependent activity contributes to efficiently dissolve these reversible structures after stress release. In contrast, Cdc19^{AFBP} and Cdc19^{irrev} amyloids fail to recruit Hsp104 and Ssa2, and consequently cannot disassemble after stress release. In *cdc19^{irrev}* cells, Hsp104 and Ssa2 remain dispersed throughout the cytoplasm. Since substrate binding of Hsp104 is ATP-dependent [56], this effect is likely due to the low ATP levels after stress in these cells. As expected, co-localization with Cdc19 amyloids is restored in *cdc19^{irrev} tps1Δ* cells, where ATP production can be resumed. Surprisingly, however, Hsp104 and Ssa2 fail to co-localize with irreversible amyloids formed by Cdc19^{AFBP}, even when ATP-production is restored by overexpressing Pyk2. In these mutant cells, both Hsp104 and Ssa2 form cytoplasmic puncta upon stress, as they are also needed to dissolve other aggregates formed by misfolded proteins, but do not co-localize with Cdc19. Chaperones recognize misfolded proteins by the exposure of hidden hydrophobic domains or specific sequences. Our results suggest that the conformational change triggered by FBP binding to Cdc19 amyloids not only initiates release of individual Cdc19 monomers,

but also forms a binding site for efficient chaperone recruitment. Indeed, an algorithm predicting potential Hsp70-binding sites [57] identifies a likely chaperone binding site in Cdc19, which corresponds to the FBP-binding site. We conclude that Cdc19 amyloids are physiological targets of Hsp104 and Ssa2, but FBP-binding is required to help chaperone recruitment and enhance Cdc19 amyloid re-solubilization *in vivo*. Future work will be required to explore mechanistic differences in recognition and activity of chaperones towards physiological and pathological targets.

Re-solubilization of Cdc19 amyloids restores ATP production to allow SG disassembly

Together, our results suggest the following multi-step process to disassemble Cdc19 amyloids and SGs (Fig. 5). Stressed cells suffer from low ATP levels, and ATP synthesis is a pre-requisite for SG disassembly and restart of growth-promoting processes [19]. Thus, upon stress-release, increasing FBP levels and binding to Cdc19 amyloids triggers a conformational change, which initiates disassembly and increases catalytic activity of Cdc19 to start converting the readily available PEP into ATP. FBP binding together with increasing ATP allows recruitment of the chaperones Hsp104 and Ssa2, which further disentangle Cdc19 fibrils, resulting in even more ATP production. This positive feedback loop rapidly solubilizes Cdc19 monomers, which in the presence of increasing FBP concentrations assemble into fully active tetramers [4]. The need for ATP production by Cdc19 can be bypassed with non-glycolytic substrates, which are metabolized by oxidative phosphorylation in mitochondria. While Hsp104 was identified as the critical ATP-consuming enzyme during this process [19], other cellular factors such as RNA/DNA-helicases, protein kinases and AAA-ATPases may also contribute to further dissolve SGs and restart cell growth [9]. In addition, metabolic changes can alter the physico-chemical properties of the cytoplasm, such as viscosity or crowding, which in turn may influence aggregation. For instance, recent experiments revealed that stress-induced changes in trehalose and glycogen levels tune cytoplasmic diffusion, thereby altering phase separation [15]. While our results suggest that regulated trehalose levels contribute to Cdc19 disassembly *in vivo*, this effect is rather minor compared to FBP binding to Cdc19 amyloids. Nevertheless, breakdown of trehalose upon stress release decreases cytosolic viscosity and releases glucose-6-phosphate to increase glycolytic flux

and further boost FBP and ATP production [58]. Finally, high ATP levels may also directly function as biological hydrotrope, and together with increasing cytosolic pH facilitate SG solubilisation [27].

Conceptually, our results reveal a mechanism whereby the metabolic and energetic state of the cell is coupled to SG disassembly *via* the regulated formation of Cdc19 amyloids. With this work, we highlight the crucial function of reversible Cdc19 amyloids in SG regulation and cell survival under stress, and elucidate how functional amyloids can be disassembled in a physiological context. As several metabolic enzymes reversibly aggregate during stress [59], similar mechanisms may regulate their activity. Finally, although the interplay between physiological and pathological amyloids remains to be explored, understanding how amyloids can be regulated in a cellular context might provide important insights into understanding the pathogenesis of neurodegenerative disease.

Methods

Media, growth assays, yeast strains and plasmid construction

All plasmids and yeast strains used in this work are listed in Supplementary Tables S1 and S2, respectively. Yeast strains and plasmids were obtained using standard molecular biology protocols, and details are available upon request. Yeast cells were grown at 30 °C in synthetic SD media (2% glucose, 0.5% NH₄-sulfate, 0.17% yeast nitrogen base, and amino acids) or YPD media (2% C-source, 2% peptone, 1% yeast extract), as previously described [4]. Where indicated, 2% glucose in SD media was substituted with 2% ethanol. When grown exponentially, Cdc19^{irrev} strains were never allowed to grow above an optical density 600 (OD₆₀₀) of 0.8 to avoid stress conditions. Heat shock was induced by shifting exponentially growing cells (OD₆₀₀ 0.4-0.6) for 30 min to 42 °C. Wherever indicated, cycloheximide (Sigma-Aldrich, C-7698), was added to the medium to a final concentration of 25 µg/ml. Growth rates of different strains before or after stress were determined by measuring OD₆₀₀ in a 96-well plate (Greiner Bio-One) using a plate reader (BioLector m2p-labs or ClarioStar BMG Labtech) over several hours at 30 °C, or by spotting dilution series of those cells onto appropriate SD plates and incubating them for 3 days at 30 °C. Overexpression of proteins from estradiol-inducible promoters was

achieved by exposing cells to 10 mM estradiol (Sigma-Aldrich E8875) for 3 hours, and Hsp104 activity was inhibited with 5 mM Gdn-HCl (Sigma-Aldrich 50950) for 3 h prior to stress initiation.

Fluorescence microscopy, image analysis and quantification

Fluorescence microscopy was performed using a Nikon Eclipse Ti-E microscope with Micro-Manager software. For time-lapse experiments, cells were loaded in commercial microfluidic chips (CellASIC ONIX2, Merck Millipore) at 30 °C as previously described [4]. Heat shock was induced thanks to the chip's temperature controller by increasing the temperature to 42 °C for 30 min, before returning it to 30 °C, or by shifting cultures for 30 min in a shaking incubator (Kühner shaker, LT W Lab-Therm) pre-heated at 42 °C (the two heat shock methods proved to be equivalent). Images were recorded every 10 min. Quantifications were carried out as described in the corresponding figure legends.

Screen for suppressors of irreversible Cdc19 aggregates

Cells expressing Cdc19^{irrev}-GFP were grown in SD-His media to OD₆₀₀ of 0.6, heat shocked for 30 min at 42 °C in a shaking incubator (Kühner shaker, LT W Lab-Therm), collected by centrifugation and spread on SD-His plates to single colonies. Surviving single colonies were isolated and visually inspected by fluorescence microscopy. Pyk2 was deleted by homologous recombination in promising candidates to exclude suppressors that upregulate Pyk2. Remaining suppressors were subsequently backcrossed to wild-type and dissected to ensure Mendelian segregation of suppressors. Suppressor strains with single gene traits were further characterized and subjected to whole-genome sequencing to identify relevant mutations. For this, 10 HIS positive heat shock-resistant spores and 10 HIS positive heat shock-sensitive spores were pooled to minimize sequencing errors and secondary mutations. Identified mutations in single genes were validated by crossing *cdc19^{irrev}* strains with the full-deletion of the mutated gene.

Protein purification

Plasmids expressing wild-type or mutant Cdc19-strep constructs were transformed into *E. coli* cells (Rosetta). Cells were grown at 37 °C in LB media (1% peptone, 0.5% yeast extract, 0.5% NaCl)

with 30 µg/ml chloramphenicol and 100 µg/ml carbenicillin to OD₆₀₀ 0.6. Protein production was induced by adding 0.1 mM IPTG to the cells, which were then grown at 16 °C overnight. Cells were harvested by centrifugation, resuspended in pre-cooled lysis buffer (100 mM Tris/HCl pH 7.4, 200 mM NaCl, 1 mM MgCl₂, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), protease inhibitor mix (Roche) and 75 U/ml of Pierce universal nuclease), and processed for lysis by freezer milling (SPEX SamplePrep 6870 Freezer/Mill; five cycles of 2 min cooling and 2 min grinding at setting 15 CPS). Extracts were cleared by centrifugation at 4 °C for 30 min at 48000 g, and the supernatant was loaded on a Strep-Tactin Superflow Plus column (Qiagen) at 4 °C following the manufacturer's instructions. Proteins were eluted using 2.5 mM D-desthiobiotin in purification buffer (100 mM Tris/HCl pH 7.4, 200 mM NaCl, 1 mM MgCl₂, 10% glycerol, 1 mM DTT, 1 mM PMSF). Fractions were collected, their purity was checked by SDS-PAGE and Coomassie blue staining, and pure fractions were pooled and aliquoted for storage at -80 °C until further use.

Transmission electron microscopy (TEM)

After thawing on ice, Cdc19 samples in purification buffer pH 7.4 were centrifuged at 21000 g and 4 °C for 10 min and the supernatant with soluble Cdc19 was retrieved. A 0.2 mg/ml Cdc19 dilution was then prepared in purification buffer pH 7.4 and the pH was adjusted to 6-7. Subsequently, a non-glow discharged carbon film 300 mesh copper grid (CF300-CU from Electron Microscopy Sciences) was submerged in this Cdc19 dilution and incubated for 10 min at 42 °C. Excess sample was then manually removed with Whatman filter paper. Where indicated, grids with pre-formed Cdc19 aggregates were incubated with 4 mM FBP or 4 mM F6P dissolved in purification buffer for 20 min, or simply washed twice with purification buffer, and stained with two drops of 2% uranyl acetate. Transmission electron micrographs were acquired with a FEI Morgagni 268 microscope at 100 kV using a CCD 1376 × 1032 pixel camera at different magnifications.

***In vitro* protein-metabolite precipitation**

Purified wild-type or mutant Cdc19 was thawed on ice and cleared by centrifugation (4 °C, 10 min, 21000 g). Different metabolites (FBP, F6P ATP, PEP, trehalose) were first solubilized in the same

buffer as the protein (100 mM Tris/HCl pH 7.4, 200 mM NaCl, 1 mM MgCl₂, 10% glycerol, 1 mM DTT, 1 mM PMSF) and were then individually added to Cdc19 to the indicated final concentrations. Final protein concentration was 0.5 mg/ml. Protein-metabolite samples were incubated for 14 h at 30 °C. Aggregated Cdc19 was pelleted by centrifugation (4 °C, 10 min, 21000 g), and separated from the supernatant containing soluble Cdc19. Aggregation was quantified by loading pellet and supernatant on a SDS-PAGE gel, followed by Coomassie blue staining and quantification with ImageJ software.

***In vitro* aggregate re-solubilization with FBP**

Purified wild-type Cdc19 was heat shocked for 10 min at 42 °C and the resulting aggregates were incubated with buffer or 20 mM FBP for 5 h at 4 °C. Aggregates were pelleted by centrifugation (4 °C, 10 min, 21000 g) and Cdc19 re-solubilization in the presence or absence of FBP was quantified by loading supernatant and pellet fractions on a SDS-PAGE gel, followed by Coomassie blue staining.

Metabolite extraction and LC-MS/MS measurements

For metabolite extraction, 1 ml culture sample was vacuum-filtered on a 0.45 µm pore size PVDF filter (Millipore) and the filter containing the cell pellet was immediately transferred into 4 ml of -20 °C cold extraction solution [40:40:20 acetonitrile/methanol/water, HPLC-grade solvents] supplemented with 200 µl of ¹³C internal standard and incubated at -20 °C for 2 h. Afterwards, the extraction solution was transferred to 15 ml tubes on ice, centrifuged for 15 min at 0 °C, 4000 rpm and the supernatant containing the metabolites was transferred to fresh tubes. Samples were dried in a speed-vac overnight and stored at -80 °C until analysis. Prior to targeted metabolomics measurements, samples were resuspended in 100 µl ddH₂O.

Compounds were analyzed using an LC-MS/MS mass spectrometer system consisting of a 1290 Infinity LC (Agilent Technologies) coupled to a 5500 QTRAP triple quadrupole mass spectrometer (AB Sciex) in negative multiple reaction monitoring (MRM) scan mode. Five µl of metabolite extracts were injected on an Agilent Poroshell 120 HILIC-Z column (150 x 2.1 mm, 2.7 µm; Agilent, Santa Clara, CA) using a mobile phase A (water, 10 mM ammonium acetate, 5 µM medronic acid, pH 9) and mobile phase B (90% acetonitrile, 10% water, 10 mM ammonium acetate, 5 µM medronic acid, pH 9)

at a constant flow rate of 250 μ l/min. Gradient was as follows: 10% A, 2 min: 10% A, 12 min: 40% A, 15 min: 40% A, 16 min: 10% A, 24 min: 10% A. The MRM settings were adapted from Yuan *et al.* [60]. The raw data were processed and analyzed using custom software in Matlab (Mathworks).

Intracellular metabolite concentration was calculated based on the measured $^{12}\text{C}/^{13}\text{C}$ ratio, assuming a dry weight [61] of 0.53 g*1-l*OD-1 and a cell volume [62] of 0.002 l*g-1. Calibration curves were obtained from dilutions of pure compounds containing same amounts of ^{13}C internal standard.

Protein extraction and Western blotting

Proteins were precipitated using a TCA/acetone-based protocol. Briefly, exponentially growing cells were incubated 10 min on ice after the addition of 10% TCA, centrifuged (5 min, 4 °C, 21000 g), and pellets were washed twice with acetone. Precipitated proteins were resuspended using a SDS-based loading buffer and protein levels were quantified by Western blotting with specific antibodies. The following antibodies were used (all at 1:3000 dilution): α -GFP (Roche, 11 814 460 001), rabbit polyclonal α -Crm1 antibody (provided by Karsten Weis), α -Pgk1 (Invitrogen, 459250), HRP-coupled secondary antibody (Biorad, 170-6516) and α -Hsp104 (1:1000) (Abcam, ab69549).

Pyruvate kinase activity assay

Pyruvate kinase activity was measured with an enzymatic assay where pyruvate production by Cdc19 is coupled to lactate production by lactate dehydrogenase and the simultaneous conversion of NADH to NAD⁺ is measured spectrophotometrically at 340 nm. Briefly, purified Cdc19 was diluted to 0.2 mg/ml and either kept on ice or heat shocked 60 min at 42 °C. Soluble or aggregated Cdc19 was diluted in an activity buffer (50 mM imidazole pH 7, 100 mM KCl, 25 mM MgCl₂, 10 mM ADP, 0.3 mM NADH, 10 U/ml LDH) to a final protein concentration of 2 μ g/ml. Reactions were started by adding PEP to a final concentration of 2 mM, and decrease in absorbance at 340 nm was monitored over time.

Size-exclusion chromatography (SEC)

Purified wild-type or mutant Cdc19 was thawed on ice and cleared from possible aggregates by centrifuging 10 min at 21000 g at 4 °C. Protein (0.1 mg) was loaded on a Superdex 200 10/300 GL size-exclusion column (GE Healthcare) connected to an ÄKTA pure (GE Healthcare) at 4 °C. UV absorbance (280 and 215 nm) was measured to follow elution of proteins as a function of elution volume, and fractions were collected, run on a SDS-PAGE gel, and stained with Coomassie blue.

Lysates for size-exclusion chromatography were prepared by growing cells expressing wild-type or mutant Cdc19-GFP in SD media to an OD₆₀₀ of 0.6. Cells were harvested by centrifugation, and pellets were resuspended in lysis buffer (PBS buffer (pH 7.4), 10% glycerol, 0.5% Tween 20, 20 mM β-glycero-phosphate, 1 mM vanadate, 1 mM NaF, 1 mM PMSF, protease inhibitor mix (Roche)). Cells were mixed with glass beads (Sigma-Aldrich, 425-600 μm) and lysed with a FastPrep homogenizer (FastPrep-24 5G, MP Biochemicals). Resulting extracts were cleared first by centrifugation (10 min at 21000 g at 4 °C), and then by filtering (0.2 μm filter, Millipore). Protein concentration of the supernatant was measured by Nanodrop and a total of 4 mg protein was loaded on a Superdex 200 10/300 GL size-exclusion column (GE Healthcare) connected to an ÄKTA pure (GE Healthcare). The column had been previously equilibrated in lysis buffer and run according to manufacturer's instructions. Fractions were collected and analyzed by Western blotting with an α-GFP antibody.

Circular dichroism (CD), Thioflavin T (ThT) and Congo Red (CR) staining *in vitro*

CD spectra of purified wild-type or mutant Cdc19 (0.2 mg/ml) were measured with a J-710 spectropolarimeter (Jasco) using a quartz cuvette with 0.1 cm path length.

For *in vitro* measurements with the amyloid dyes, Thioflavin T (ThT, Sigma-Aldrich, T3516) or Congo Red (CR, Sigma-Aldrich, 75768-25MG) were dissolved in purification buffer (100 mM Tris/HCl pH 7.4, 200 mM NaCl, 1 mM MgCl₂, 10% glycerol, 1 mM DTT, 1 mM PMSF) to a final concentration of 2.5 mM or 1 mM, respectively, and filtered (0.2 μm, Millipore). Purified wild-type or mutant Cdc19 were thawed on ice, cleared by centrifugation (10 min, 4 °C, 21000 g), diluted to 0.3 mg/ml in purification buffer, and then either heat shocked (10 min, 42 °C) or kept on ice. ThT or CR solution was then added (1:10 dilution) to the heat shock or control samples, and fluorescence

intensity was measured in a 384-well plate (Corning Life Sciences) at the indicated temperature using a CLARIOstar plate reader (BMG Labtech). For ThT, excitation was set at 450 nm, and emission spectra were recorded at 490 nm. For CR, fluorescence intensity was measured at 614 nm with excitation at 560 nm.

Congo Red (CR) staining *ex vivo*

For *ex vivo* CR staining, cells expressing wild-type or mutant Cdc19-GFP were collected by centrifugation and resuspended in lysis buffer (PBS buffer pH 7.4, 10% glycerol, 0.5% Tween 20, 1 mM PMSF). Glass beads (Sigma-Aldrich, 425–600 μ m) were added and lysis was performed at 4 °C with a FastPrep homogenizer (FastPrep-24 5G, MP Biochemicals). Lysates were cleared by centrifuging 2 min at 2000 rpm at 4 °C, and total protein concentration was determined by Nanodrop. Glass microscope slides were cleaned with consecutive washes of H₂O, 100% ethanol and acetone, and carefully dried. Then, 3 mm-wide stripes of double-sided tape were applied along the length of the glass slides in order to form three channels (approximately 3 mm-wide), which were then covered with a cover slip. Assembled flow chambers were functionalized by incubating them with a solution of 0.3 mg/ml Avidin DN (Vector Laboratories) in T50 buffer (10 mM Tris pH 8.0 and 50 mM NaCl) for 20 min, then blocking with 0.1% puronic F-127 (Sigma-Aldrich) in T50 buffer for 30 min, washing twice with 0.1 mg/ml BSA in T50 buffer, and incubating with biotinylated G-protein in lysis buffer for 30 min. Finally, 10 nM of anti-GFP antibody in lysis buffer were flushed into the channels and total protein extract was loaded in each channel. Sample was allowed to bind for 10 min and then washed three times with lysis buffer to remove non-bound excess. For staining, a solution of 250 μ M CR in lysis buffer was flushed in the channels, and excess was removed by washing twice with lysis buffer. Fluorescence microscopy was performed as described above.

LiP-MS experiments, SRM assay development and quantification analysis.

For the *in vivo* LiP-MS experiment, cells expressing Cdc19^{WT}-GFP or Cdc19^{irrev}-GFP were grown in SD media and three biological replicates were collected when cells were growing exponentially (OD₆₀₀ 0.6) or following entry into stationary phase (two days). Cells were resuspended

in 100 mM HEPES, 150 mM KCl, 1 mM MgCl₂, pH 7.6 and disrupted by bead beating in the presence of acid-washed glass beads in three consecutive rounds of 30 s of beating and 4 min of incubation at 4 °C. Protein concentration in the yeast extracts was determined by the bicinchoninic acid assay (BCA Protein Assay Kit, Thermo Scientific). Based on the protein concentration determined by the BCA assay, equal amounts of the cell lysates were treated with proteinase K (Sigma Aldrich) at a concentration lysate:proteases of 100:1 for three min. Then, the protease activity was quenched by heating at 95 °C and by the addition of 5% sodium deoxycholate (Sigma Aldrich) as described in [63]. Equal amounts of cell lysates were treated in parallel with vehicle instead of proteinase K to estimate Cdc19 protein amount variations in the two conditions assayed (exponential growth vs stationary phase). Tris(2-carboxyethyl)phosphine-HCl (TCEP-HCl, Thermo Fisher Scientific) was added to a final concentration of 5 mM (35 min, 37 °C) to reduce the disulfide bridges, followed by the alkylation of free cysteine residues with iodoacetamide (Sigma aldrich) at 20 mM final concentration (30 min, room temperature). Peptide and protein fragments were then further digested to completion in denaturing conditions with LysC and trypsin proteases. Before the addition of LysC and trypsin, the reaction mixtures were diluted with 0.1 M ammonium bicarbonate (Sigma Aldrich) to reach a sodium deoxycholate concentration lower than 1%. First, LysC digestion was performed at an enzyme to substrate (E/S) ratio of 1:100 for 4 h at 37 °C, then trypsin digestion was carried out at an E/S ratio of 1:100, for 16 h at 37 °C. For the *in vitro* experiment, limited proteolysis experiments were carried out with recombinantly purified Cdc19^{WT} and Cdc19^{irrev} instead of protein lysates. Aliquots of recombinantly purified Cdc19^{WT} and Cdc19^{irrev} were incubated for 10 min at 25 °C or 42 °C in triplicates to induce Cdc19 structural variations just before the limited proteolysis step.

The development and validation of Selected Reaction Monitoring (SRM) assays to measure abundances of Cdc19 was guided by a spectral library generated in-house through a LC-MS/MS analysis of the *in vivo* yeast samples on a QE-plus (Thermo Fisher scientific) mass spectrometer equipped with a nano-electrospray ion source. On-line chromatographic separation of the peptides was achieved with an Easy-nLC 1000 nano liquid chromatography system (Thermo Fisher scientific) equipped with a 40 cm fused-silica column (New Objective), packed in-house with Reprosil Pur C18

Aq, 1.9 μ m beads (Dr Maisch). The peptide mixtures (~1 μ g) were separated with a linear gradient from 5% to 35% acetonitrile in 120 min. The collected spectra were searched against the *S. cerevisiae* UniProt reference database with Sorcerer-Sequest (Thermo Electron). Trypsin was set as the digesting protease with the tolerance of two missed cleavages and semi tryptic termini. Carbamidomethylation of cysteines (+57.0214 Da) was defined as a fixed modification, and oxidation of methionine (+15.99492) as a variable modification. Protein identifications were filtered with a false discovery rate of <1%, calculated on the basis of a target-decoy approach. Proteome discoverer 2.2 (Thermo Fisher scientific) was used to build a consensus spectral library using the LiP-MS *in vivo* samples.

Peptides matching to the spectral library were ranked by intensity using Skyline (v4.1, MacCoss Lab Software, University of Washington), and the 10 most intense transitions consisting of doubly or triply charged precursor ions and singly or doubly charged fragment ions of the y- and b-ion series were experimentally tested in SRM mode to select the most suitable transitions for quantification experiments. Samples were measured on a triple quadrupole/ion-trap mass spectrometer (5500 QTrap, ABSciex) equipped with a nano-electrospray ion source and operated in SRM mode. On-line chromatographic separation of the peptides was achieved with an Eksigent 1D-plus Nano liquid chromatography system equipped with a 15 cm fused-silica column with 75 μ m inner diameter, packed in-house with Reprosil Pur C18 Aq, 5 μ m beads (Dr Maisch). The peptide mixtures (~1 μ g) were separated with a linear gradient from 5% to 35% acetonitrile in 30 min. SRM analysis was conducted with Q1 and Q3 operated at unit resolution (0.7 m/z half-maximum peak width) with a dwell time of 20 ms and a cycle time <3.0 s.

SRM-MS data was analyzed with Skyline, and for the final SRM assay retention times were scheduled and at least three transitions per precursor ion of Cdc19 were retained. The identity of each SRM peak was confirmed against the spectral library by matching realigned retention times and relative fragment-ion intensities. 199 transitions matching to 44 Cdc19 precursors were selected for Cdc19 peptide quantification purposes. Integration of peak areas was manually inspected and relative quantification was performed for the Cdc19 peptides in the LiP-MS *in vitro* and *in vivo* experiments. Statistical significance analysis was performed with MSstats (www.msstats.org). Precursor intensities

were normalized using the option “*equalize medians*” of MSstats. In the LiP-MS *in vivo* experiment peptide intensities were corrected for variations of Cdc19 protein levels between the exponential and stationary conditions. The normalization factor applied was equivalent to the relative variation of the Cdc19^{WT} or Cdc19^{irrev} proteins between the exponential and stationary conditions.

Statistical analysis and data availability

All shown data are representative results from at least three independent experiments, unless differently specified in the respective figure legend. Whenever possible, mean $\pm$ S.E.M. and individual data points of three or more independent experiments are shown. See figure legends for details. GraphPad Prism was used to analyse and plot data. Detailed experimental procedures and additional data supporting the findings of this study are available from the corresponding authors upon request.

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Author contributions

Conceptualization: GC, RD, MP; *Formal analysis:* MP; *Investigation:* GC, CW, VK, MD, AA, IP, HY, SS; *Writing – Original Draft Preparation:* GC, MP; *Writing – Review & Editing:* GC, MP, with input from all authors; *Visualization:* GC, MP; *Supervision:* MP, PP, US, AD; *Funding Acquisition:* MP, PP, US, AD. The authors declare that they have no competing financial interest related to this paper.

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Figure 1 - SG disassembly depends on re-solubilization of Cdc19 amyloids and restoration of ATP production

Figure 1: SG disassembly depends on re-solubilization of Cdc19 amyloids and restoration of ATP production

(A) Exponentially growing Cdc19^{WT}-GFP (top panel) and Cdc19^{irrev}-GFP (bottom panel) cells expressing the SG marker Pab1-CFP were grown in a microfluidic chip at 30 °C in SD-full medium (left column), subjected to heat stress for 30 min at 42 °C (middle column) and allowed to recover at 30 °C (right column). Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and merged (bottom row) to show co-localization of Cdc19 aggregates and SGs (arrow heads). Note that upon heat shock, Cdc19^{WT}-GFP forms reversible aggregates that co-localize with SGs. The mutant Cdc19^{irrev}-GFP instead forms irreversible aggregates, which prevent SG disassembly after stress release. Images are representative of three independent experiments. Scale bar: 5 µm.

(B) – (C) Cdc19^{WT} (top panels) or Cdc19^{irrev} (bottom panels) proteins were expressed and purified from *E. coli*, and imaged by negative staining transmission electron microscopy (TEM) **(B)** or analyzed by circular dichroism (CD) **(C)** before (soluble) or after a 10 min heat shock at 42 °C (aggregated). Representative images of three independent experiments are shown. Note that upon heat shock Cdc19 assembles into β -sheet-rich, filamentous amyloids. Scale bar in **(B)**: 2 µm.

(D) The catalytic activity of purified Cdc19^{WT} was measured using a lactate dehydrogenase-coupled activity assay before (soluble) or after a 30 min heat shock at 42 °C (aggregated). The bars show Cdc19 activity expressed as the decrease in absorbance at 340 nm per min. Mean, standard error of the mean (S.E.M.) and single data points of three independent experiments are indicated (statistical analysis with two-tailed t-test, $P = 0.0018$).

(E) *cdc19^{irrev}* cells are unable to re-activate Cdc19 and resume ATP production after stress release. Intracellular ATP and phosphoenolpyruvate (PEP) concentrations (µM) were measured in wild-type and *cdc19^{irrev}* cells, after these cells were subjected to a 30 min heat shock at 42 °C and allowed to recover at 30 °C for 60 min. Mean and S.E.M. determined by five independent experiments are shown (statistical analysis with Mann-Whitney test, $P_{ATP} = 0.0079$, $P_{PEP} =$

0.0079). Note that wild-type cells have high ATP levels and low PEP levels, indicating active Cdc19, while *cdc19^{irrev}* cells are not able to consume PEP and resume ATP production.

(F) Schematic drawing showing a yeast cell before, during, and after heat stress. Upon stress, Cdc19 (represented in green) forms inactive aggregates, which colocalize with SGs. ATP levels rapidly decrease under these conditions, while Cdc19's substrate phosphoenolpyruvate (PEP) accumulates. Upon stress release, Cdc19 re-solubilizes, gets re-activated, and consumes PEP to produce ATP, concomitant with SG disassembly.

(G) Serial dilutions of exponentially growing cells expressing Cdc19^{WT}-GFP or Cdc19^{irrev}-GFP and harboring where indicated a plasmid overexpressing Pyk2 from the constitutive *TEF*-promoter (Pyk2OE) were spotted on agar plates before (upper panel) or after a 30 min heat shock at 42 °C (lower panel). The plates were incubated at 30 °C and imaged after 3 days.

(H) – (I) ATP production using Cdc19-independent pathways allows Cdc19^{irrev} aggregate and SG re-solubilization after stress release. **(H)** Cells expressing Cdc19^{irrev}-GFP, the SG marker Pab1-CFP, and a plasmid overexpressing Pyk2 from the constitutive *TEF*-promoter (Pyk2OE) were grown at 30 °C in SD-full medium, subjected to heat stress for 30 min at 42 °C (left column) and allowed to recover at 30 °C (middle and right columns) in SD-full medium with 25 µg/ml cycloheximide to avoid *de novo* protein synthesis. **(I)** Cells expressing Cdc19^{irrev}-GFP and the SG marker Pab1-CFP were grown in SD medium containing 2 % ethanol as the sole carbon source at 30 °C, heat shocked for 30 min at 42 °C (left column) and allowed to recover at 30 °C (middle and right columns) in SD-full medium with 25 µg/ml cycloheximide to avoid *de novo* protein synthesis. Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and also merged (bottom row) to show co-localization of Cdc19 aggregates and SGs (arrow heads). Scale bar: 5 µm.

Figure 2 - Re-solubilization of Cdc19^{irrev} amyloids and cell cycle restart after stress are regulated by the glycolytic metabolite FBP

Figure 2: Re-solubilization of Cdc19^{irrev} amyloids and cell cycle restart after stress are regulated by the glycolytic metabolite FBP

(A) Schematic representation of glycolysis and trehalose metabolism. The enzymes and metabolites discussed in the following panels are highlighted. Abbreviations: glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-bisphosphate (FBP), phosphoenolpyruvate (PEP), trehalose-6-phosphate (T6P), hexokinase (Hxk), trehalose synthase (Tps) and phosphofructokinase (Pfk). Note that T6P inhibits the Hxk activity (dashed blunt arrow), while FBP activates Cdc19 (dashed pointed arrow).

(B) Blocking trehalose metabolism overcomes stress-induced growth arrest of *cdc19^{irrev}* cells. Serial dilutions of exponentially growing wild-type, *tps1Δ*, *tps2Δ* and *tps3Δ* cells expressing as indicated Cdc19^{WT} or Cdc19^{irrev}. An equal number of cells was spotted on agar plates before (left) or after a 30 min heat shock at 42 °C (right), and grown at 30 °C for 3 days.

(C) Blocking trehalose metabolism allows re-solubilization of Cdc19^{irrev} aggregates and SG disassembly after stress. Exponentially growing *cdc19^{irrev} tps1Δ* cells expressing the SG marker Pab1-CFP were grown in a microfluidic chip at 30 °C in SD-full medium (left column), heat shocked for 30 min at 42 °C (middle column) and allowed to recover at 30 °C (right column). Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and also merged (bottom row) to show co-localization of Cdc19 aggregates and SGs (arrow heads). Data shown are representative of three independent experiments. Scale bar: 5 μm.

(D) Intracellular ATP and phosphoenolpyruvate (PEP) concentrations (μM) were measured in wild-type, *cdc19^{irrev}*, and *cdc19^{irrev} tps1Δ* cells, after these cells were subjected to a 30 min heat shock at 42 °C and allowed to recover at 30 °C for 60 min. Mean and S.E.M. determined by five independent experiments are shown (statistical analysis with Mann-Whitney test, for *cdc19^{WT}* – *cdc19^{irrev}* comparison: $P_{ATP} = 0.0079$, for *cdc19^{irrev}* - *cdc19^{irrev} tps1Δ* comparison: $P_{ATP} = 0.0317$, $P_{PEP} = 0.0556$). Note that deletion of *tps1* in *cdc19^{irrev}* cells allows to resume ATP production after stress release.

(E) – (F) Intracellular disaccharide (E) and FBP (F) concentrations (μM) were measured by mass-spectrometry in *cdc19^{irrev}* or *cdc19^{irrev} tps1 Δ* strains before, during and after a 30 min heat shock at 42 °C. Mean and S.E.M. determined by five independent experiments are shown (statistical analysis with Mann-Whitney test, for *cdc19^{irrev}* 30 °C – Heat shock comparison: $P = 0.0317$, for *cdc19^{irrev} - cdc19^{irrev} tps1 Δ* Recovery 60 min comparison: $P = 0.0317$). Note that intracellular disaccharides in yeast almost exclusively consist of trehalose [64]. Thus, in *tps1 Δ* cells trehalose production is impaired and FBP accumulates.

(G) Serial dilutions of exponentially growing wild-type, *pfk2 Δ* , *tps1 Δ* or *tps1 Δ pfk2 Δ* cells expressing either Cdc19^{WT} or Cdc19^{irrev} were spotted on agar plates before (left) or after a 30 min heat shock of 42 °C (right). The plates were imaged after 3 days at 30 °C.

(H) Exponentially growing *tps1 Δ pfk2 Δ* cells expressing the SG marker Pab1-CFP and the GFP-tagged Cdc19^{irrev} mutant were grown in a microfluidic chip at 30 °C in SD-full medium (left column), subjected to heat stress for 30 min at 42 °C (middle column) and allowed to recover at 30 °C (right column). Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and also merged (bottom row) to show co-localization of Cdc19 aggregates and SGs (arrow heads). The intensity of the GFP and CFP images before heat shock has been slightly reduced to allow better visualization. Scale bar: 5 μm . Note that deleting the FBP-producing enzyme Pfk2 not only abolishes growth restart observed in *tps1 Δ* cells, but also Cdc19^{irrev} aggregate re-solubilization and SG disassembly.

Figure 3 - FBP directly regulates formation and disassembly of reversible Cdc19 amyloids

Figure 3: FBP directly regulates formation and disassembly of reversible Cdc19 amyloids

(A) Schematic representation of how FBP may regulate Cdc19 amyloid formation and disassembly.

FBP binds soluble Cdc19 and promotes its tetramerization. Since Cdc19 tetramers are less aggregation-prone than the monomers [4], FBP may help to maintain a soluble Cdc19 pool, thus reducing aggregate formation (dashed arrow 1). Alternatively, FBP may also bind Cdc19 amyloids and promote aggregate re-solubilization (dashed arrow 2). Note that the two mechanisms are not mutually exclusive, and likely co-operate to achieve efficient Cdc19 regulation.

(B) – (D) Binding of FBP to soluble Cdc19^{WT} reduces its aggregation *in vitro*. Purified wild-type

Cdc19 **(B)**, FBP binding-deficient Cdc19^{ΔFBP} mutant **(C)** or Cdc19^{irrev} mutant **(D)** proteins were mixed with 5 mM FBP or for control 5 mM F6P or buffer and incubated at 30 °C for 14 hours. Cdc19 aggregates were separated from soluble Cdc19 by centrifugation and the supernatant (Sup, containing soluble Cdc19) and pellet (Pellet, containing aggregated Cdc19) fractions were analysed by SDS-PAGE and Coomassie blue staining (left gels). Cdc19 amount was quantified in the Pellet (left graphs) and Supernatant (right graphs) by measuring Cdc19 band intensity using ImageJ and normalizing it to buffer control, and is shown as graphs with mean ± S.E.M. from three independent experiments (statistical analysis with two-tailed t-test, *P* = 0.0342).

(E) – (F) Cdc19^{irrev} is exclusively monomeric *in vivo* and *in vitro*. **(E)** Extracts from cells expressing

GFP-tagged Cdc19^{WT} (top panel) or Cdc19^{irrev} (bottom panel) were separated by size-exclusion chromatography (SEC) and selected peak fractions were analysed by Western blot with an α-GFP antibody. The elution volume (ml) is indicated below, and the numbers on the left indicate size markers (in kDa). **(F)** Recombinant wild-type (Cdc19^{WT}), Cdc19^{irrev} or for control tetrameric Cdc19^{ΔPEP} mutant proteins were purified and analysed by SEC. Absorbance at 215 nm (arbitrary units) was normalized and plotted as a function of elution volume (ml). Data are representative of three independent experiments.

(G) Direct FBP binding to Cdc19 is necessary to re-solubilize Cdc19 amyloids *in vivo*. Wild-type or *tps1Δ* cells expressing GFP-tagged wild-type Cdc19 (top row) or the FBP binding-defective Cdc19^{ΔFBP} mutant (middle and bottom row) and overexpressing Pyk2 were grown in a microfluidic chip at 30 °C in SD-full medium, subjected to heat stress for 30 min at 42 °C (left column) and allowed to recover at 30 °C in the presence of 25 μg/ml cycloheximide to avoid *de novo* protein synthesis. Representative GFP-images were taken 60 (middle column) or 240 min (right column) after heat shock end. Cdc19-GFP aggregates are marked with arrowheads. Scale bars: 5 μm.

(H) Binding of FBP to pre-formed Cdc19^{WT} amyloids triggers conformational changes visible by negative staining transmission electron microscopy (TEM) using uranyl acetate stain. Purified Cdc19^{WT} (upper row) or the FBP binding-defective Cdc19^{ΔFBP} mutant were aggregated by a 10 min heat shock at 42 °C, and the resulting amyloids were incubated with FBP (4 mM) or for control F6P (4 mM) or buffer. Scale bar: 500 nm. Arrows mark FBP-induced “bulges” that are frequently observed along the fibrils axis. The indicated sections (dashed squares) of representative fibrils were enlarged 1.5 times to better visualize the distinct morphologies (inserts). Images are representative of three independent experiments.

Figure 4 - FBP binding to Cdc19 amyloids promotes chaperone recruitment triggering Cdc19 re-solubilization and SG disassembly *in vivo*

Figure 4: FBP binding to Cdc19 amyloids promotes chaperone recruitment triggering Cdc19 re-solubilization and SG disassembly *in vivo*

(A) – (B) Inhibition of Hsp104 activity delays re-solubilization of Cdc19^{WT} and Cdc19^{irrev} aggregates in wild-type and *tps1Δ* cells, respectively. Wild-type and *tps1Δ* cells expressing respectively GFP-tagged wild-type Cdc19 or the Cdc19^{irrev} mutant were treated with 5 mM of the Hsp104 inhibitor Gdn-HCl for 3 hours prior to a 30 min heat shock at 42 °C. **(A)** Aggregate disassembly after stress release was monitored at the indicated time points by fluorescence microscopy. Representative images of three independent experiments are shown, with arrowheads marking Cdc19 aggregates. Scale bar: 5 μm. **(B)** Cdc19 aggregate disassembly was quantified by counting the number of foci at different time points after stress. The average time to reach a 50% reduction in foci number is displayed. Error bars represent S.E.M. (statistical analysis with two-tailed t-test, $P_{WT} = 0.0268$, $P_{irrev+tps1Δ} = 0.048$).

C) – (D) Overexpression of Hsp104 partially rescues growth of *cdc19^{irrev}* cells after heat shock. **(C)** Serial dilutions of exponentially growing wild-type or *tps1Δ* cells expressing either Cdc19^{WT} or Cdc19^{irrev} were spotted on agar plates before (left) or after a 30 min heat shock of 42 °C (right). Where indicated, overexpression of Hsp104 was induced by treating cells with 10 mM estradiol for 3 hours. The plates were imaged after 3 days at 30 °C. **(D)** Growth restart of the indicated strains treated as described above was also quantified by measuring the cell density (OD₆₀₀) over time after inoculation of equal cell numbers at 30 °C. Mean cell density 22 hours after stress release is shown, with error bars representing S.E.M. from three independent experiments (statistical analysis with two-tailed t-test, $P_{irrev-irrev+tps1Δ} < 0.0001$, $P_{irrev+tps1Δ-irrev+tps1Δ+Hsp104OE} = 0.0055$). Note that Hsp104 overexpression and increased FBP levels co-operate to rescue *cdc19^{irrev}* cells after heat shock.

(E) – (G) FBP binding to Cdc19 amyloids promotes chaperone recruitment, which correlates with aggregate disassembly. Wild-type or *tps1Δ* cells co-expressing mCherry-tagged Hsp104 **(E, G)** or Ssa2 **(F)** and either GFP-tagged Cdc19^{WT}, Cdc19^{irrev} or Cdc19^{AFBP} were subjected to heat stress for 30 min at 42 °C and imaged by fluorescence microscopy. The *cdc19^{AFBP}* cells **(G)**

overexpress Pyk2 to restore ATP production and cell viability. Representative GFP- (left column) and mCherry-images (middle column) are shown, together with the merged image (right column) to visualize co-localization of Cdc19 aggregates and these chaperones. Images are representative of three independent experiments. Scale bar: 5 μ m. The percentage (%) of cells where Cdc19 and Hsp104 or Ssa2 co-localized was quantified and plotted as mean with S.E.M. in the bar graph. At least 300 cells were analysed for each condition.

Figure 5 - Molecular network coupling cellular metabolism with reversible Cdc19 amyloids and stress granule dynamics

Figure 5: Molecular network coupling cellular metabolism with reversible Cdc19 amyloids and stress granule dynamics

The metabolite FBP couples cellular metabolism to the assembly state and activity of Cdc19, which in turn regulates SG dynamics. During cell growth, FBP is produced through glycolysis and its binding to Cdc19 promotes the formation of active, non-aggregation-prone Cdc19 tetramers. Upon stress, FBP levels rapidly drop, leading to tetramer disassembly and the accumulation of Cdc19 monomers. In this state, Cdc19 exposes its aggregation-prone LCR and is primed to form Cdc19 amyloids, which are inactive and accumulate in SGs. After stress release, FBP levels rise and FBP directly binds to Cdc19 amyloids, thereby triggering a conformational change along the Cdc19 fibrils that facilitates the recruitment of Hsp104 and Ssa2. These chaperones allow the re-solubilisation of Cdc19 amyloids and the release of Cdc19 monomers. Re-solubilised, active Cdc19 in turn uses PEP to produce ATP, which is required to activate chaperones to further solubilize Cdc19 and increase energy production. This positive feedback loop fuels disassembly of Cdc19 amyloids and other SG components. Concomitant breakdown of trehalose releases glucose moieties for glycolysis and modulates cytoplasmic diffusion. Orchestrated disassembly of SGs then releases several mRNAs and metabolic enzymes, and allows full resumption of cell growth and cell cycle progression.

Supplementary Figure 1 - Cdc19 aggregates are amyloids both *in vitro* and *in vivo*

Suppl. Figure 1: Cdc19 aggregates are amyloids both *in vitro* and *in vivo*

(A) Schematic representation of the domains of Cdc19. The catalytic domain, FBP-binding domain, and the aggregation-prone low complexity region (LCR) are highlighted. The four mutated residues located within the LCR in the Cdc19^{irrev} mutant are indicated (red circles).

(B) - (C) Both Cdc19^{WT} and Cdc19^{irrev} form ThT- and Congo Red-positive aggregates upon heat shock *in vitro*. Purified Cdc19^{WT}, Cdc19^{irrev} and a non-aggregating Cdc19 mutant as negative control (Cdc19^{ΔPEP} [1]) were incubated with ThT (B) or Congo Red (C) at 4 °C or after a 10 min heat shock at 42 °C, and fluorescence emission was measured at 490 nm or 614 nm, respectively. The graphs show the mean ± S.E.M. and individual data points of three independent experiments (statistical analysis with two-tailed t-test, for ThT: $P_{WT} < 0.0001$, $P_{irrev} = 0.0091$, for Congo Red: $P_{WT} = 0.0003$, $P_{irrev} = 0.0128$).

(D) *In vivo*-formed Cdc19^{WT} and Cdc19^{irrev} aggregates are stained with Congo Red. Cells expressing GFP-tagged Cdc19^{WT} or Cdc19^{irrev} were harvested when exponentially growing or after a 30 min heat shock at 42 °C. Cells were gently lysed as described in Materials and Methods, Cdc19-GFP was immobilized in a GFP-trap microfluidic chamber and then stained with Congo Red. GFP (left column) and the Congo Red signal (middle column) were detected by fluorescence microscopy, and merged to visualize co-localization (right column). Cdc19-GFP aggregates stained with Congo Red are marked with arrow heads. Images are representative of three independent experiments.

(E) Limited-Proteolysis Mass Spectrometry (LiP-MS) results indicate that Cdc19^{WT} and Cdc19^{irrev} undergo comparable structural transitions upon aggregation *in vitro* and *in vivo*. Recombinant Cdc19^{WT} or Cdc19^{irrev} were purified and soluble Cdc19 or heat shock-induced Cdc19 aggregates (10 min at 42 °C) were analysed by LiP-MS as described in Material and Methods. Volcano plots were used to compare Cdc19 peptides detected in soluble and aggregated Cdc19^{WT} and Cdc19^{irrev}, and upregulated (red) or downregulated (blue) conformation-sensitive peptides are highlighted. To compare these *in vitro* data with *in vivo* conformations, cells expressing Cdc19^{WT}-GFP or Cdc19^{irrev}-GFP were harvested during exponential growth or stationary phase

(2 days) to induce aggregation. Cells were lysed and the extracts analysed by LiP-MS as described in Material and Methods. The volcano plots were used to compare Cdc19 peptides detected during exponential growth and starvation, and upregulated (red) or downregulated (blue) conformation-sensitive peptides are highlighted. The conformation-specific LiP-MS-peptides detected in the *in vitro* and *in vivo* experiments were mapped to the Cdc19 protein, schematically represented with the functional domains as described in panel A. Note that the conformation-specific LiP-MS peptides are comparable between wild-type and the Cdc19^{irrev} mutant, with similar aggregate structures assembled *in vitro* upon heat shock and *in vivo* upon starvation.

Supplementary Figure 2 - Genetic screening identifies trehalose metabolism as a regulator of reversible Cdc19 aggregation

Suppl. Figure 2: Genetic screening identifies trehalose metabolism as a regulator of reversible Cdc19 aggregation

(A) Schematic representation of the screening protocol used to identify suppressors of stress-induced growth arrest in *Cdc19^{irrev}* cells. *cdc19^{irrev}* cells were subjected to a 30 min heat shock at 42 °C, then plated at 30 °C, and the few spontaneous suppressors which could resume growth after stress were isolated. *pyk2* was deleted in these cells and single gene suppressors still able to restart cell growth after stress were further characterized. Whole-genome sequencing was performed to identify responsible mutations.

(B) The *cdc19^{irrev}* suppressor screen identified a single point mutation (G385D) in the *tps3* gene encoding for a subunit of trehalose synthase. Serial dilutions of exponentially growing *cdc19^{WT}*, *cdc19^{irrev}* and *cdc19^{irrev} tps3-G385D* were spotted on agar plates before (left) or after a 30 min heat shock of 42 °C (right). The plates were imaged after 3 days at 30 °C.

(C) Exponentially growing *tps3-G385D* cells expressing GFP-tagged *Cdc19^{irrev}* and the SG marker Pab1-CFP were grown in a microfluidic chip at 30 °C in SD-full medium (left column), heat shocked for 30 min at 42 °C (middle column) and allowed to recover at 30 °C (right column). Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and merged (bottom row) to visualize co-localization of *Cdc19^{irrev}* aggregates and SGs (arrow heads). Scale bar: 5 μm.

(D) Serial dilutions of exponentially growing wild-type, *tps1Δ*, *tps2Δ*, and *tps3Δ* cells were spotted on agar plates before (left) or after a 30 min heat shock of 42 °C (right). The plates were imaged after 3 days at 30 °C.

(E) Wild-type cells accumulate high levels of disaccharides upon heat shock, which almost exclusively consist of trehalose [2]. Deletion of either *tps1* or *tps2* abolishes this increase. Intracellular disaccharide concentrations (μM) were measured by mass-spectrometry in wild-type, *tps1Δ*, and *tps2Δ* cells at the indicated time points before, during and after a 30 min heat shock at 42 °C. Mean ± S.E.M. determined by five (for wild-type) and three (for *tps1Δ* and

tps2Δ) independent experiments are shown (statistical analysis with Mann-Whitney test, $P = 0.0079$).

(F) Extracts prepared from wild-type, *tps1Δ*, *pfk2Δ* or *tps1Δ pfk2Δ* expressing either GFP-tagged Cdc19^{WT} or Cdc19^{irrev} were immunoblotted with antibodies against GFP (left panel) or for control Pgk1 (right panel). The mean Cdc19-GFP levels normalized with Pgk1 are shown ($n = 2$). Note that neither *tps1Δ* nor *pfk2Δ* significantly alter Cdc19 protein levels.

(G) Exponentially growing *tps2Δ* cells expressing GFP-tagged Cdc19^{irrev} and the SG marker Pab1-CFP were grown in a microfluidic chip at 30 °C in SD-full medium (left column), heat shocked for 30 min at 42 °C (middle column) and allowed to recover at 30 °C (right column). Representative GFP- (top row) and CFP-images (middle row) were taken at the indicated time points, and merged (bottom row) to visualize co-localization of Cdc19^{irrev} aggregates and SGs (arrow heads). Scale bar: 5 μm.

(F) Intracellular disaccharide (left) and FBP (right) concentrations (μM) were measured by mass-spectrometry in *cdc19^{irrev}* and *cdc19^{irrev} tps2Δ* strains at the indicated time points before, during and after a 30 min heat shock at 42 °C. Mean ± S.E.M. determined by four (for *cdc19^{irrev} tps2Δ*) and five (for *cdc19^{irrev}*) independent experiments are shown (statistical analysis with Mann-Whitney test, $P_{Disaccharides} = 0.0159$, $P_{FBP} = 0.0317$). Note that intracellular disaccharides in yeast almost exclusively consist of trehalose [2], and that *cdc19^{irrev} tps2Δ* have low trehalose and low FBP levels during and after stress.

Supplementary Figure 3 - FBP specifically reduces aggregation of purified Cdc19^{WT}, but not Cdc19^{ΔFBP} and Cdc19^{irrev} mutant proteins

Suppl. Figure 3: FBP specifically reduces aggregation of purified Cdc19^{WT}, but not Cdc19^{ΔFBP} and Cdc19^{irrev} mutant proteins

(A) – (C) Purified wild-type Cdc19 (A), FBP binding-deficient Cdc19^{ΔFBP} mutant (B) or Cdc19^{irrev} mutant (C) proteins were mixed as indicated with 5 mM FBP or for control 5 mM F6P, 5 mM ATP, 5 mM PEP, 5 mM trehalose or buffer and incubated at 30 °C for 14 hours. Cdc19 aggregates were separated from soluble protein by centrifugation and the supernatant (Sup, containing soluble Cdc19) and pellet (Pellet, containing aggregated Cdc19) fractions analysed by SDS-PAGE and Coomassie blue staining (left gels). Cdc19 amount was quantified in the Pellet (left graphs) and Supernatant (right graphs) by measuring Cdc19 band intensity using ImageJ and normalizing to buffer controls, and is displayed as mean and S.E.M. from three independent experiments (statistical analysis with two-tailed t-test, $P = 0.0342$). Note that, in contrast to Cdc19^{ΔFBP} and Cdc19^{irrev}, FBP reduces aggregation of Cdc19^{WT}, while all other tested metabolites have no effect on aggregation.

(D) Addition of FBP alone is not sufficient to re-solubilize pre-formed Cdc19^{WT} amyloids *in vitro*. Purified Cdc19^{WT} (Input) was incubated for 10 min at 42 °C to trigger its aggregation, and the resulting amyloids were incubated with (20 mM) or without (0 mM) FBP for several hours. Cdc19 re-solubilization was then tested by centrifugation and analysis of the resulting supernatant (Sup) and pellet (Pellet) fractions by SDS-PAGE and Coomassie blue staining. The image is representative of three independent experiments.

Supplementary Figure 4 - The chaperones Hsp104 and Ssa2 co-operate with FBP to efficiently disassemble Cdc19 amyloids *in vivo*

Suppl. Figure 4: The chaperones Hsp104 and Ssa2 co-operate with FBP to efficiently disassemble Cdc19 amyloids *in vivo*

(A) Co-overexpression of Hsp104 and Ssa2 partially restores growth of *cdc19^{irrev}* cells after heat stress. *cdc19^{WT}* and *cdc19^{irrev}* cells overexpressing as indicated Hsp104 or Ssa2, or both together by addition of 10 mM estradiol for 3 hours were treated for 30 min at 42 °C. Serial dilutions were spotted on agar plates and grown at 30 °C for 3 days. The image is representative of three independent experiments.

(B) Hsp104 protein levels were quantified in the indicated strains by Western blot either in the absence (-) or presence (+) of 10 mM estradiol for 3 hours. The image is representative of three independent experiments.

(C) Increased FBP levels and Hsp104 co-operate to efficiently restart growth after stress in *cdc19^{irrev}* cells. Exponentially growing wild-type or *tps1Δ* cells expressing either Cdc19^{WT} or Cdc19^{irrev} were subjected to a 30 min heat shock at 42 °C. Where indicated, overexpression of Hsp104 was induced by treating cells with 10 mM estradiol for 3 hours. Growth restart after stress release of the indicated strains was quantified by measuring the cell density (OD600) over time after inoculation of equal cell numbers at 30 °C. Mean cell density 22 hours after stress release is shown, with error bars representing S.E.M. from three independent experiments. Note that Hsp104 overexpression and increased FBP levels co-operate to rescue *cdc19^{irrev}* cells after heat shock.

(D) Wild-type (left and middle panels) or *tps1Δ* (right panels) cells co-expressing mCherry-tagged Hsp104 and either GFP-tagged Cdc19^{WT} (left panel) or Cdc19^{irrev} mutant (middle and right panels) were heat stressed for 30 min at 42 °C and imaged by fluorescence microscopy at the times indicated (minutes). Representative GFP- (left row) and mCherry-images (middle row) are shown, together with the merged image (bottom row) to visualize co-localization of Cdc19 aggregates and Hsp104. Images are representative of three independent experiments. Scale bar: 5 μm.

Strain name	Genotype	Source
<i>cdc19Δ</i> + Pyk2OE	BY4741, <i>cdc19Δ::NAT^R</i> ; <i>pRS416-pTEF1-PYK2</i>	Saad et al.
Cdc19 ^{WT} -GFP	BY4741, <i>CDC19::CDC19-GFP-HIS3</i>	Invitrogen
Cdc19 ^{WT} -GFP + <i>tps1Δ</i>	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>tps1Δ::KAN^R</i>	This study
Cdc19 ^{WT} -GFP + <i>tps2Δ</i>	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>tps2Δ::KAN^R</i>	This study
Cdc19 ^{WT} -GFP + <i>pfk2Δ</i>	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>pfk2Δ::KAN^R</i>	This study
Cdc19 ^{WT} -GFP + Pyk2OE	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i>	Saad et al.
Cdc19 ^{WT} -GFP + Hsp104-mCherry	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>HSP104::HSP104-mCherry</i>	This study
Cdc19 ^{WT} -GFP + Ssa2-mCherry	BY4741, <i>CDC19::CDC19-GFP-HIS3</i> ; <i>SSA2::SSA2-mCherry</i>	This study
Cdc19 ^{irrev} -GFP	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i>	Saad et al.
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps1Δ::KAN^R</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + <i>pfk2Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps1Δ::NAT^R</i> ; <i>pfk2Δ::KAN^R</i>	This study
Cdc19 ^{irrev} -GFP + Pyk2OE	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i>	This study
Cdc19 ^{irrev} -GFP Original suppressor	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps3-G386D</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps3Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps3Δ::KAN^R</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps2Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps2Δ::KAN^R</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps2Δ</i> + <i>pfk2Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps2Δ::NAT^R</i> ; <i>pfk2Δ::KAN^R</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + <i>tps2Δ</i>	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps1Δ::KAN^R</i> ; <i>tps2Δ::NAT^R</i>	This study
Cdc19 ^{irrev} -GFP + Hsp104-mCherry	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>HSP104::HSP104-mCherry</i>	This study
Cdc19 ^{irrev} -GFP + Ssa2-mCherry	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>SSA2::SSA2-mCherry</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + Hsp104-mCherry	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps1Δ::KAN^R</i> ; <i>HSP104::HSP104-mCherry</i>	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + Ssa2-mCherry	BY4741, <i>CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3</i> ; <i>tps1Δ::KAN^R</i> ; <i>SSA2::SSA2-mCherry</i>	This study
Cdc19 ^{ΔFBP} -GFP	BY4741, <i>CDC19::CDC19-T402E-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i>	Saad et al.
Cdc19 ^{ΔFBP} -GFP + <i>tps1Δ</i>	BY4741, <i>CDC19::CDC19-T402E-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i> ; <i>tps1Δ::KAN^R</i>	This study
Cdc19 ^{ΔFBP} -GFP + Hsp104-mCherry	BY4741, <i>CDC19::CDC19-T402E-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i> ; <i>HSP104::HSP104-mCherry</i>	This study
Cdc19 ^{ΔFBP} -GFP + <i>tps1Δ</i> + Hsp104-mCherry	BY4741, <i>CDC19::CDC19-T402E-GFP-HIS3</i> ; <i>pRS416-pTEF1-PYK2</i> ; <i>tps1Δ::KAN^R</i> ; <i>HSP104::HSP104-mCherry</i>	This study

Cdc19 ^{Not-agg} -GFP	BY4741, CDC19::CDC19-R49A-GFP- HIS3; pRS416-pTEF1-PYK2	Saad et al.
Cdc19 ^{irrev} -GFP + Hsp104OE	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3 HSP104::HSP104-mCherry	This study
Cdc19 ^{irrev} -GFP + Ssa2OE	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; SSA2::SSA2-mCherry	This study
Cdc19 ^{irrev} -GFP + Hsp104OE + Ssa2OE	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; SSA2 <i>Gal</i> <i>GST</i> ; Hsp104OE	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + Hsp104OE	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps1Δ</i> ::KAN ^R ; Hsp104OE	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + Hsp104OE + <i>SsaeOE</i>	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps1Δ</i> ::KAN ^R ; Hsp104OE	This study
Cdc19 ^{WT} -GFP + Pab1-CFP	BY4741, CDC19::CDC19-GFP-HIS3; pRS415-Pab1-CFP	Saad et al.
Cdc19 ^{WT} -GFP + <i>tps1Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-GFP-HIS3; <i>tps1Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{WT} -GFP + <i>tps2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-GFP-HIS3; <i>tps2Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{WT} -GFP + <i>pfk2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-GFP-HIS3; <i>pfk2Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{WT} -GFP + Pab1-CFP + Pyk2OE	BY4741 CDC19::CDC19-GFP-HIS3; pRS416-pTEF1-PYK2; pRS415-Pab1-CFP	This study
Cdc19 ^{Irrev} -GFP + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; pRS415- Pab1-CFP	Saad et al.
Cdc19 ^{Irrev} -GFP + <i>tps1Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps1Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{irrev} -GFP + <i>tps3Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps3Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{irrev} -GFP + <i>tps2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps2Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{irrev} -GFP + <i>tps1Δ</i> + <i>tps2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps1Δ</i> ::KAN ^R ; <i>tps2Δ</i> ::NAT ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{Irrev} -GFP + <i>tps1Δ</i> + <i>pfk2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps1Δ</i> ::NAT ^R ; <i>pfk2Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{irrev} -GFP + <i>tps2Δ</i> + <i>pfk2Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps2Δ</i> ::NAT ^R ; <i>pfk2Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study
Cdc19 ^{Irrev} -GFP + Pyk2OE + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; pRS416- pTEF1-PYK2; pRS415-Pab1-CFP	This study
Cdc19 ^{Irrev} -GFP Original suppressor + Pab1-CFP	BY4741, CDC19::CDC19-T372D, T376D, S377D, S385D-GFP-HIS3; <i>tps3</i> - <i>G386D</i> ; pRS415-Pab1-CFP	This study
Cdc19 ^{ΔFBP} -GFP + Pab1-CFP	BY4741, CDC19::CDC19-T402E-GFP-HIS3 ; pRS416-pTEF1-PYK2; pRS415- Pab1-CFP	Saad et al.
Cdc19 ^{ΔFBP} -GFP + <i>tps1Δ</i> + Pab1-CFP	BY4741, CDC19::CDC19-T402E-GFP-HIS3 ; pRS416-pTEF1-PYK2; <i>tps1Δ</i> ::KAN ^R ; pRS415-Pab1-CFP	This study

130 **Suppl. Table S2:** Plasmids used in this study

Plasmid name	Genotype	Source
Cdc19 ^{WT}	<i>pRS413-pTEF1-CDC19</i>	Grüning et al.
Pyk2OE	<i>pRS416-pTEF1-PYK2</i>	Saad et al.
Cdc19 ^{WT} -GFP	<i>pRS413-pTEF1-CDC19-GFP</i>	Saad et al.
Cdc19 ^{ΔFBP} -GFP	<i>pRS413-pTEF1-CDC19-T402E-GFP</i>	Saad et al.
Cdc19 ^{Not-agg.} -GFP	<i>pRS413-pTEF1-CDC19-R49A-GFP</i>	Saad et al.
Cdc19 ^{irrev} -GFP	<i>pRS413-pTEF1-CDC19-T372D, T376D, S377D, S385D-GFP</i>	Saad et al.
GFP	<i>pRS416-pGPD-GFP</i>	Saad et al.
Pab1-CFP	<i>pRS415-pPAB1-PAB1-CFP</i>	Saad et al.
Cdc19 ^{WT} -strep _{x2}	<i>pET17b-CDC19-Strep_{x2}</i>	Saad et al.
Cdc19 ^{Not-agg.} -strep _{x2}	<i>pET17b-CDC19-R49A-Strep_{x2}</i>	Saad et al.
Cdc19 ^{irrev} -strep _{x2}	<i>pET17b-CDC19-T372D, T376D, S377D, S385D-Strep_{x2}</i>	This Study
Cdc19 ^{ΔFBP} -strep _{x2}	<i>pET17b-CDC19-T402E-Strep_{x2}</i>	This study

Figures

Figure 1

G disassembly depends on re-solubilization of Cdc19 amyloids and restoration of ATP production (see Manuscript File for full figure legend)

Figure 2

Re-solubilization of Cdc19^{irrev} amyloids and cell cycle restart 901 after stress are regulated by the glycolytic metabolite FBP (see Manuscript File for full figure legend)

Figure 3

FBP directly regulates formation and disassembly of reversible Cdc19 amyloids(see Manuscript File for full figure legend)

Figure 4

FBP binding to Cdc19 amyloids promotes chaperone recruitment triggering Cdc19 resolubilization and SG disassembly in vivo(see Manuscript File for full figure legend)

Figure 5

Molecular network coupling cellular metabolism with reversible Cdc19 amyloids and stress granule dynamics(see Manuscript File for full figure legend)

Supplementary Files

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- [SupplementaryTable1Strains.xlsx](#)
- [SupplementaryTable2Plasmids.xlsx](#)

- [SupplementaryFigure1Cereghettietai.ai](#)
- [SupplementaryFigure2Cereghettietai.ai](#)
- [SupplementaryFigure3Cereghettietai.ai](#)
- [SupplementaryFigure4Cereghettietai.ai](#)