UC Davis
UC Davis Previously Published Works

Title
Human CTP synthase filament structure reveals the active enzyme conformation

Permalink
https://escholarship.org/uc/item/3rw467w9

Journal
Nature Structural & Molecular Biology, 24(6)

ISSN
1545-9993

Authors
Lynch, Eric M
Hicks, Derrick R
Shepherd, Matthew
et al.

Publication Date
2017-06-01

DOI
10.1038/nsmb.3407

Peer reviewed
Human CTP synthase filament structure reveals the active enzyme conformation

Eric M Lynch1, Derrick R Hicks1,2, Matthew Shepherd3, James A Endrizzi4, Allison Maker1, Jesse M Hansen1,5, Rachael M Barry6, Zemer Gitai6, Enoch P Baldwin4, & Justin M Kollman1

The universally conserved enzyme CTP synthase (CTPS) forms filaments in bacteria and eukaryotes. In bacteria, polymerization inhibits CTPS activity and is required for nucleotide homeostasis. Here we show that for human CTPS, polymerization increases catalytic activity. The cryo-EM structures of bacterial and human CTPS filaments differ considerably in overall architecture and in the conformation of the CTPS protomer, explaining the divergent consequences of polymerization on activity. The structure of human CTPS filament, the first structure of the full-length human enzyme, reveals a novel active conformation. The filament structures elucidate allosteric mechanisms of assembly and regulation that rely on a conserved conformational equilibrium. The findings may provide a mechanism for increasing human CTPS activity in response to metabolic state and challenge the assumption that metabolic filaments are generally storage forms of inactive enzymes. Allosteric regulation of CTPS polymerization by ligands likely represents a fundamental mechanism underlying assembly of other metabolic filaments.

Many metabolic enzymes undergo dynamic rearrangement into large-scale cellular structures in response to specific metabolic cues; a subset form defined filamentous structures in cells1–7. While the enzymes involved are generally well characterized by extensive study of their catalytic mechanisms, monomer structures, and regulation, the filamentous forms remain largely uncharacterized. Where functional data do exist, however, it is clear that metabolic filaments are important for regulating enzyme activity and for maintaining cellular homeostasis8–12.

Filamentous polymers of CTPS appear to be universally conserved, having been observed in bacteria, yeast, flies, and human cells1–3,13. CTPS is a focal point for regulation of pyrimidine levels, as it directly converts UTP to CTP. We recently showed that in bacteria the product CTP drives assembly of CTPS into a filament form with lower activity8, consistent with the role of CTP as an allosteric inhibitor of CTPS. The polymer is sensitive to substrate–product balance and can rapidly depolymerize into active tetramers in response to changes in substrate concentration. These dynamics buffer the catalytic activity of CTPS to maintain a defined ribonucleotide balance. Importantly, disruption of bacterial CTPS polymerization significantly affects cell growth and metabolism, indicating that polymerization is essential for cellular homeostasis. However, the precise molecular mechanisms of CTP-induced polymerization and inhibition of CTPS have remained unclear. Moreover, while CTPS filaments appear to be universally conserved, it is unknown whether the molecular mechanisms and functional consequences of polymerization are the same across kingdoms.

CTPS directly catalyzes the conversion of UTP to CTP. The enzyme is a homotetramer, with each monomer composed of a glutamine amidotransferase (GAT) domain and a kinase-like ammonia-ligase (AL) domain and joined by an α-helical linker14. Ammonia generated in the GAT domain is transferred to the AL domain and then ligated to UTP to form CTP in an ATP-hydrolysis-dependent reaction, with the allosteric regulator GTP playing a role in coupling the reactions. The mechanism of ammonia transfer remains unclear, although a conformational change that alters the relative orientations of the two catalytic domains has been proposed to open an ammonia channel between the active sites15. Feedback inhibition occurs through CTP binding at a site that partially overlaps with the UTP substrate-binding site16. The catalytic mechanism, domain structures, and the tetrameric quaternary structure of the enzyme are broadly conserved between eukaryotes and bacteria14,15,17,18.

Given that the overall structures, catalytic mechanisms, and cellular polymerization of CTPS are conserved from bacteria to humans, it has been widely assumed that the mechanisms and functional consequences of polymerization are conserved for CTPS across species19. However, very little is known about the function of CTPS polymerization in eukaryotes beyond the observation that it appears to be part of a cellular stress response and to vary with developmental stage in some organisms4,9,10,20,21. Moreover, recent cell biological studies of CTPS polymerization in eukaryotes came to opposite conclusions about the effects of assembly on enzyme activity5,11.

Here, we use cryo-EM, X-ray crystallography, and kinetic assays to study the allosteric regulation of human and Escherichia coli CTPS...
filaments. We demonstrate that human CTPS filaments assemble in the presence of substrates, have increased catalytic activity, and reveal a novel active conformation of CTPS. Further, we show that both human and E. coli CTPS undergo a conserved conformational cycle controlled by substrate and product binding but have opposite determinants for filament assembly. Given the importance of human CTPS as a target for cancer and immunosuppressive drugs and of bacterial CTPS as a target for antiparasitic treatments, these molecular insights into CTPS structure and allostery may provide novel opportunities to disrupt its function and regulation.

**RESULTS**

**Structure of the ecCTPS filament**

To elucidate the mechanism of CTP-induced filament assembly in E. coli CTPS (ecCTPS), we determined the structure of CTP- and ADP-bound ecCTPS filaments at 4.6-Å resolution by cryo-EM (Fig. 1, Table 1 and Supplementary Fig. 1). The ecCTPS filament consists of stacked tetramers that interact through their GAT and linker domains. The tetramer interface is reorganized relative to previous ecCTPS tetramer crystal structures in the apo conformation, bringing the CTPS subunits into closer contact around the bound CTP. It was unclear, however, whether this novel ecCTPS conformation was a cause or consequence of filament assembly.

To distinguish between these possibilities, we solved a new ecCTPS–CTP co-crystal structure (Table 2) that reveals a tetramer with quaternary packing nearly identical to that which we observe in the filament (Fig. 1e). The fact that we observe the same tetramer conformation in the filament and in a crystal without filament-assembly contacts supports the notion that CTP binding allosterically controls ecCTPS assembly by directly inducing a filament-competent conformation of ecCTPS.

The conformational changes between this novel tetramer conformation and the apo state are almost entirely rigid-body motions, with the subunits in each state nearly the same (<0.5 Å r.m.s. deviation) but with one exception: helix 218–228 is shifted 3.6 Å closer to the CTP bound on an adjacent monomer so that Phe227 packs against the CTP base. This shift also repositions Asn229 at the base of helix 218–228, creating a new hydrogen-bond network with Arg158 and Glu155 across the tetramer interface, likely stabilizing the filament conformation (Supplementary Fig. 2). We previously reported that E155K, a mutation associated with drug resistance through relief of CTPS inhibition, cannot assemble filaments. Our new structure suggests this glutamate-to-lysine mutation interferes with the hydrogen-bond network that occurs across the tetramer interfaces in the inhibited conformation. These changes alter the relative orientations of the polymerization interfaces and position them to allow assembly only in the CTP-bound state (Fig. 1f, Supplementary Videos 1 and 2).

**Allosteric inhibition of ecCTPS in the filament**

We next sought to establish the mechanism of polymerization-induced inhibition of ecCTPS. We considered three possibilities: that substrates are occluded from the active site by assembly contacts, that the filament sterically blocks a conformational change necessary for ammonia transfer, and that the conformation of CTPS in the filament is allosterically inhibitory.

To test these possibilities, we first sought to decouple ecCTPS polymerization from CTP binding by engineering cysteine disulfide crosslinks at filament-assembly interfaces (ecCTPSCC). Under nonreducing conditions, ecCTPSCC spontaneously polymerizes without nucleotides, and its structure is identical to the wild-type structure minus

---

**Figure 1** Mechanism of ecCTPS assembly. (a) Cryo-EM reconstruction of ecCTPS filament at 4.6-Å resolution. (b) A single ecCTPS tetramer from the filament, colored by protomer (blue and green) and nucleotide density highlighted in orange. (c) The inhibitory CTP binding site in apo-ecCTPS with CTP soaked into the crystals (PDB 2AD5). (d) The inhibitory CTP binding site in the cryo-EM structure shows compaction of the tetramer around CTP. (e) Overlay of the filament structure (gray) with the structure of ecCTPS co-crystallized with CTP (color). Arrows indicate the rotation of subunits relative to the apo conformation in (c). (f) Overlay of the apo-ecCTPS crystal structure (gray) with the CTP-bound filament structure (color). The protomers on the lower right are superposed, revealing a rotation of 5.5° and a 4-Å translation in the positions of the protomers on the upper left. (g) Filament assembly contacts between ecCTPS tetramers can only be made in the novel CTP-bound conformation, as superposition of two apo tetramers would result in backbone clashing at the GAT contact site.
nucleotides (Fig. 2a–d). Under reducing conditions, unassembled ecCTPSCC is as active as the wild-type enzyme, but apo-ecCTPSCC filaments preassembled under nonreducing conditions exhibit a fivefold reduction in activity relative to free tetramers, indicating that filament assembly directly inhibits enzymatic activity (Fig. 2e).

When products (CTP and ADP) are soaked into preassembled ecCTPSCC filaments, clear density is observed for the nucleotides in cryo-EM reconstructions, ruling out nucleotide occlusion as a mechanism of inhibition (Fig. 2f). To test whether filaments impair ammonia transfer between active sites, we measured the activity of ecCTPSCC filaments after adding ammonia directly as a substrate. Under these conditions, ecCTPSCC exhibited the same reduction in activity as the wild type, indicating that inhibition within the filament is not solely the result of an inability to couple the GAT and AL reactions. Finally, we tested whether ecCTPSCC filaments could bind substrates (UTP and ATP) by soaking preassembled filaments with saturating concentrations of nucleotides. Under these conditions, no density is observed for UTP in the active site (Fig. 2f), suggesting reduced affinity for substrates in the filament conformation. Together, these results indicate that polymerization allosterically regulates ecCTPSC activity by stabilizing an intrinsically lower-activity state upon incorporation into filaments, independent of CTP binding.

Assembly and activity of hCTPS polymer

Surprisingly, we found that purified human CTP synthase 1 (hCTPS1) polymerizes in the presence of UTP and ATP substrates but not in the presence of CTP and ADP products, exactly the opposite behavior to that observed with ecCTPS (Fig. 3a). hCTPS1 filaments were stable in the presence of UTP, ATP, and GTP, but they disassembled over time upon addition of glutamine, presumably as a result of accumulation of CTP product from the complete synthesis reaction (Supplementary Fig. 3b,c). Indeed, adding CTP directly to filaments assembled with substrates also resulted in disassembly, while filaments assembled with UTP, the nonhydrolyzable ATP analog AMP-PNP, and GTP were stable following glutamine addition, owing to a lack of CTP synthesis (Supplementary Fig. 3b,c).

Table 1  EM data collection and refinement statistics

| Data collection | ecCTPS filament (EMD-8504, PDB 5U3C) | hCTPS1 filament (EMD-8474, PDB 5U05) | ecCTPS tetramer (EMD-8475, PDB 5U05) | ecCTPS-CC (products) (EMD-8513, PDB 5U6R) | ecCTPS-CC (substrates) (EMD-8491) | ecCTPS-CC (apo) (EMD-8490) | hCTPS1-H355A tetramer (EMD-8476) |
|-----------------|--------------------------------------|--------------------------------------|---------------------------------------|----------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Electron microscope | Polara | TF20 | TF20 | TF20 | TF20 | TF20 | Spirit |
| Voltage (kV) | 300 | 200 | 200 | 200 | 200 | 200 | 120 |
| Electron detector | K2 summit | K2 summit | K2 summit | K2 summit | K2 summit | K2 summit | Ultrascan CCD |
| Electron dose (e−/Å²) | 34 | 68 | 45 | 45 | 45 | 45 | 30 |
| Pixel size (Å) | 1.22 | 1.26 | 1.26 | 1.26 | 1.26 | 1.26 | 2.07 |
| Reconstructor | D2 | D2 | D2 | D2 | D2 | D2 | D2 |
| Refined helical symmetry | 48.2°, 82.1 Å | 60.4°, 104.1 Å | 50.6°, 83.5 Å | 48.2°, 82.3 Å | – |
| Resolution (0.143 fsc) (Å) | 7.9 | 6.407 | 31.170 | 7.135 | 13.715 | 4.413 |
| Refinement | PDB 2AD5 | PDB 2VO1, 2VKT | PDB 2AD5 | PDB 2AD5 |
| Model composition | | | | | | |
| Protein residues | 534 | 559 | 534 | 534 |
| Ligands | CTP, ADP, Mg | UTP, ATP, Mg | CTP, ADP, Mg |
| Validation | | | |
| Clashscore | 2 | 4 | 14 | 2 |
| Poor rotamers (%) | 0.1 | 0.2 | 0.1 | 0.1 |
| Ramachandran plot | | | |
| Favored (%) | 94 | 96 | 94 | 94 |
| Allowed (%) | 4 | 4 | 4 | 4 |
| Outliers (%) | 1 | 1 | 1 | 1 |

Table 2  Data collection and refinement statistics

| Data collection | ecCTPS CTP-bound (PDB 5TKV) |
|-----------------|-----------------------------|
| Space group | P21212 |
| Cell dimensions | a, b, c (Å) 159.16, 110.68, 129.49 |
| | α, β, γ (°) 90.00, 90.00, 90.00 |
| Resolution (Å) | 30.0–2.70 (2.82–2.70) |
| Rmerge | 0.092 (0.310) |
| I/σ(I) | 11.2 (3.1) |
| Completeness (%) | 98.3 (99.0) |
| Redundancy | 3.4 (3.2) |
| Refinement | |
| Resolution (Å) | 29.8–2.70 |
| No. reflections | 59,184/3,159 |
| Rwork/Rfree | 0.158/0.208 |
| No. atoms | 8,827 |
| Protein | 8,305 |
| Ligand/ion (CTP/Mg) | 158 |
| Water | 364 |
| B factors (Å²) | Protein 46 |
| | Ligand/ion (CTP/Mg) 43 |
| | Water 47 |
| R.m.s. deviations | Bond lengths (Å) 0.0156 |
| | Bond angles (°) 1.94 |

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.
To understand how the universally conserved CTPS enzyme could have opposite determinants for filament assembly in bacteria and humans, we solved the cryo-EM structure of hCTPS1 filaments assembled in the presence of substrates UTP and ATP and the allosteric effector GTP at 6.1-Å resolution (Fig. 3b, c, Table 1, Supplementary Fig. 4). The UTP–ATP–GTP combination was selected for structure determination as it gave the most robust polymerization of hCTPS1. Like ecCTPS, hCTPS1 is composed of stacked tetramers, but beyond this, the structures differ in their assembly contacts, tetramer interfaces, and protomer conformations. hCTPS1 filament assembly is mediated primarily by an insert in the GAT domain that appeared early in eukaryotic evolution (Fig. 3d, Supplementary Fig. 5a). This GAT–GAT assembly interaction, which is also observed in the crystal packing of existing structures of isolated hCTPS2 GAT domains (Fig. 3e), uses a completely different interface from filament assembly in the E. coli enzyme and accounts for the drastic differences in filament architecture between species. There may be additional assembly contacts between C-terminal densities that are predicted to be disordered and are poorly resolved in the cryo-EM structure (Supplementary Fig. 4g). Like ecCTPS, hCTPS1 assembly is allosterically controlled, but in this case, a novel substrate-bound conformation drives polymerization.

To test the biochemical consequences of polymerization, we mutated a single conserved histidine in the hCTPS1 GAT-domain helical insert (H355A). Unlike the wild-type enzyme, hCTPS1-H355A completely failed to polymerize in the presence of UTP, ATP, and GTP (Fig. 3a, Supplementary Fig. 5b). A negative-stain reconstruction of hCTPS1-H355A under these conditions confirmed that the overall tetramer structure, which is necessary for CTPS activity, was not affected by the H355A mutation (Supplementary Fig. 5c). However, the H355A mutation led to a six-fold reduction in hCTPS1 activity (Fig. 3f), indicating that hCTPS1 activity is enhanced upon subunit assembly into filaments, likely because hCTPS1 is locked in a more active conformation. This assembly of active polymers is in contrast with the suggestion from previous work, including our own description of the ecCTPS filament, that these structures generally function as inactive reservoirs of metabolic capacity. Rather, in some instances, it seems that there may be more complex regulatory schemes in which large-scale polymerization functions to boost enzyme activity.

hCTPS filament structure
The hCTPS1 filament structure provides the first view of any CTPS in the substrate-bound active state, which differs from all existing CTPS
structures in both the tetramerization interface and the protomer conformation (Fig. 4). Density for ATP and UTP substrates is clearly visible in the cryo-EM map, indicating that the hCTPS1 filaments are primed for catalysis upon addition of glutamine (Fig. 4b). This is consistent with the observation that metabolic filaments assemble in response to glutamine deprivation in cells21. Compared with CTPS structures in the inactive state, including the crystal structure of the hCTPS1 AL-domain tetramer, which exhibits analogous packing to that of helices 221–228 and 149–164 in PDB 5TKV, the active hCTPS1 tetramer is extended along the filament axis and compressed perpendicular to the filament axis by approximately 6 Å and 5 Å, respectively (Supplementary Videos 3 and 4), owing to rearrangements of the tetramer interface (Fig. 4c).

Within each hCTPS1 protomer, there is a large conformational change arising from an approximately 10° rotation between the GAT and AL domains relative to all previously reported full-length CTPS structures (Fig. 4d,e). The rotation is necessary to position the GAT domains for polymerization. In addition, the rotation appears to align two cavities within the GAT and AL domains to form a tunnel. The resolution of our cryo-EM structure is insufficient to clearly describe the nature of this tunnel, but it may function to facilitate ammonia transfer between the two active sites, similar to ammonia tunnels described in other enzymes28 (Fig. 4f,g). This would be consistent with earlier predictions that a conformational change is required for ammonia transfer and coupling the GAT and AL reactions14,15.

**CTPS conformational states are conserved across kingdoms**

We hypothesized that the conformation observed in the hCTPS1 filament is a generally conserved active conformation of the enzyme. However, as there were no previous full-length structures of hCTPS1, we could not rule out the possibility that the unique conformation was specific to the human enzyme. To determine whether ecCTPS adopts the same conformation on substrate binding, we determined the structure of the ecCTPS tetramer bound to UTP and AMP-PNP by cryo-EM at 8 Å (Fig. 5a,b, Table 1). The structure has the same overall conformation as the active hCTPS1 filament both at the tetramer interface (Fig. 5c, Supplementary Videos 3 and 4) and in the rotation between the AL and GAT domains (Fig. 5d,e), confirming that the conformational states of CTPS are conserved from bacteria to humans.

**DISCUSSION**

The structures of human and bacterial CTPS filaments suggest a model for the allosteric regulation of CTPS polymerization. The conformational equilibrium between apo, substrate-bound, and product-bound states of the CTPS tetramer is universally conserved, while evolutionary divergence at polymerization interfaces has reversed the determinants for filament assembly. The inactive product-bound conformation is stabilized in bacterial filaments, while the active substrate-bound conformation is stabilized in eukaryotic filaments (Fig. 5f). For both filament types, polymerization may mediate a more cooperative transition between high- and low-activity states, providing more switch-like...
behavior in response to small changes in substrate and product concentrations. What remains unclear is the relative advantage of stabilizing hCTPS in an active conformation in the polymer. One possibility is that stabilizing the polymer under conditions of cellular stress keeps the bulk of hCTPS in a state primed for maximal activity immediately on return to normal growth conditions.

Both hCTPS and ecCTPS polymerize through association of their GAT domains. A short helical insertion at this interface both provides the primary assembly interface in hCTPS and prevents hCTPS from assembling with ecCTPS-like contacts (Fig. 4d,e, Supplementary Fig. 4f). The approximately ten-residue insertion appeared during early eukaryotic evolution, as only a few early-diverging single-celled lineages lack the insert. This raises the interesting question of how the two different filament forms evolved. One possibility is that CTPS of early eukaryotes formed inhibitory filaments like those of bacteria, and the appearance of the GAT insert switched the filaments to an active state. An alternative is that CTPS polymerization arose independently in bacterial and eukaryotic lineages, so that the appearance of polymerization as a deeply conserved feature of CTPS actually reflects the independent evolution of CTPS filaments. This may be the more likely scenario, as the appearance of filamentous forms of so many different metabolic enzymes in many different lineages suggests that this is a relatively common evolutionary strategy. Investigating the role of polymerization in earlier-diverging eukaryotes, many of them human pathogens, will be necessary to clarify the evolutionary history of CTPS filaments.

For the many different metabolic enzymes that form filaments, it seems likely that in many cases, like with CTPS, binding of substrates or other ligands allosterically regulates assembly. Moreover, the ability to interconvert between dispersed and polymeric enzyme forms with different intrinsic activities may provide a general mechanism for regulating or localizing metabolic activity under complex cellular conditions. For example, CTPS activity is also regulated by phosphorylation, raising the possibility that post-translational modifications could modulate CTPS activity by influencing filament assembly, shifting the equilibrium between polymers and free tetramers to tune the total level of enzyme activity, independent of substrate and product concentrations. Similarly, binding partners may influence assembly or disassembly of CTPS filaments. This would be analogous to cytoskeletal systems like actin filaments and microtubules in which substrate binding and hydrolysis drive intrinsic polymer dynamics, but multiple factors interact to influence the timing and location of assembly. Indeed, CTPS filaments co-localize with other metabolic and signaling enzymes, suggesting that cellular factors may specifically recognize and interact with metabolic polymers.

Several cell biological studies have shown that eukaryotic CTPS filaments assemble in response to nutrient stress and at particular developmental stages. Although some of these studies have
suggested that eukaryotic CTPS filaments may be composed of catalytically inactive dimers\textsuperscript{11,20}, our work here demonstrates that isolated hCTPS filaments are polymers of active substrate-bound tetramers. Consistent with our results, CTPS in Drosophila germ cells forms filaments at developmental stages with a high demand for CTP, and a constitutively active mutant of hCTPS forms filaments under conditions where the wild-type protein is diffuse throughout the cytoplasm\textsuperscript{9}. Experiments in human cells have also shown that metabolic filament assembly is required for glutamine deprivation and disassembly upon glutamine addition\textsuperscript{21}, an effect which we observe here with purified hCTPS1.

Phosphorylation at various sites is known to modulate eukaryotic CTPS activity, leading to changes in cellular concentrations of CTP and phospholipids\textsuperscript{29,31,32}. Multiple phosphorylation sites are located in the C-terminal tail of hCTPS1 (ref. 30), which may form assembly contacts in the hCTPS1 filament. It will therefore be interesting to investigate whether phosphorylation has a direct effect on hCTPS1 polymerization, and how enzyme regulation by polymerization is integrated into other regulatory pathways. The CTPS filament structures presented here will provide a mechanistic basis for future investigations of the cellular consequences of polymerization.

\section*{METHODS}
Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

\section*{ACKNOWLEDGMENTS}
The authors are grateful to G. Carman (Rutgers University) for the hCTPS1-expressing \textit{S. cerevisiae} strain. This work was supported by the US National Institutes of Health (R01 GM113996 to J.M.K.) and the Human Frontier Science Program (RGY0076/2013 to Z.G. and J.M.K.) Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research and by the National Institutes of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

\section*{AUTHOR CONTRIBUTIONS}
E.M.L. and J.M.K. wrote the manuscript. E.M.L., J.M.K., and D.R.H. solved the EM structures. J.A.E. and E.P.B. solved the crystal structure of ecCTPS. M.S. and J.M.H. generated disulfide cross-linked ecCTPS constructs and optimized purification and assembly conditions. E.M.L. and A.M. carried out CTPS activity assays. R.M.B. and Z.G. performed initial characterization of ecCTPS filaments. E.M.L., Z.G., E.P.B. and J.M.K. contributed to experimental design and data analysis and interpretation.

\section*{COMPETING FINANCIAL INTERESTS}
The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Noree, C., Sato, B.K., Broyer, R.M. & Wilhelm, J.E. Identification of novel filament-forming proteins in \textit{Saccharomyces cerevisiae} and \textit{Drosophila melanogaster}. \textit{J. Cell Biol.} \textbf{190}, 541–551 (2010).
2. Liu, J.L. Intracellular compartmentation of CTP synthase in Drosophila. \textit{J. Genet. Genomics} \textbf{37}, 281–296 (2010).
3. Ingerson-Mahar, M., Briegel, A., Werner, J.N., Jensen, G.J. & Gitai, Z. The metabolic enzyme CTP synthase forms cytoskeletal filaments. \textit{Nat. Cell Biol.} \textbf{12}, 739–746 (2010).
4. Carcamo, W.C. et al. Induction of cytoplasmic rods and rings structures by inhibition of the CTP and GTP synthetic pathway in mammalian cells. \textit{PLoS One} \textbf{6}, e29690 (2011).
5. Shen, Q.J. et al. Filamentation of metabolic enzymes in \textit{Saccharomyces cerevisiae}. \textit{J. Genet. Genomics} \textbf{43}, 393–404 (2016).
6. Narayanaswamy, R. et al. Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. \textit{Proc. Natl. Acad. Sci. USA} \textbf{106}, 10147–10152 (2009).
7. An, S., Kumar, R., Sheets, E.D. & Benkovic, S.J. Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. \textit{Science} \textbf{320}, 103–106 (2008).
8. Barry, R.M. et al. Large-scale filament formation inhibits the activity of CTP synthetase. \textit{eLife} \textbf{3}, e03638 (2014).
9. Strochlic, T.I. et al. Ack kinase regulates CTP synthase filaments during \textit{Drosophila} oogenesis. \textit{EMBO Rep.} \textbf{15}, 1184–1191 (2014).
10. Petrovska, I. et al. Filament formation by metabolic enzymes is a specific adaptation to an advanced state of cellular starvation. eLife 3, e02409 (2014).
11. Noree, C., Monfort, E., Shiau, A.K. & Wilhelm, J.E. Common regulatory control of CTP synthase enzyme activity and filament formation. Mol. Biol. Cell 25, 2282-2290 (2014).
12. Chang, C.C. et al. Cytoophidium assembly reflects upregulation of IMPDH activity. J. Cell Sci. 128, 3550–3555 (2015).
13. Chen, K. et al. Glutamine analogs promote cytoophidium assembly in human and Drosophila cells. J. Genet. Genomics 36, 591–602 (2011).
14. Endrizzi, J.A., Kim, H., Anderson, P.M. & Baldwin, E.P. Crystal structure of Escherichia coli cytidine triphosphate synthetase, a nucleotide-regulated glutamine amidotransferase/ATP-dependent amidoligase fusion protein and homologue of anticancer and antiparasitic drug targets. Biochemistry 43, 6447–6463 (2004).
15. Goto, M., Omi, R., Nakagawa, N., Miyahara, I. & Hirotsu, K. Crystal structures of CTP synthetase reveal ATP, UTP, and glutamine binding sites. Structure 12, 1413–1423 (2004).
16. Endrizzi, J.A., Kim, H., Anderson, P.M. & Baldwin, E.P. Mechanisms of product feedback regulation and drug resistance in cytidine triphosphate synthetases from the structure of a CTP-inhibited complex. Biochemistry 44, 13491–13499 (2005).
17. Kursula, P. et al. Structure of the synthetase domain of human CTP synthetase, a target for anticancer therapy. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 62, 613–617 (2006).
18. Lauritsen, I., Willemoës, M., Jensen, K.F., Johansson, E. & Harris, P. Structure of the dimeric form of CTP synthetase from Sulfolobus solfataricus. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 67, 201–208 (2011).
19. Aughey, G.N. & Liu, J.L. Metabolic regulation via enzyme filamentation. Crit. Rev. Biochem. Mol. Biol. 51, 282–293 (2016).
20. Aughey, G.N. et al. Nucleotide synthesis is regulated by cytoophidium formation during neurodevelopment and adaptive metabolism. Biol. Open 3, 1045–1056 (2014).
21. Calise, S.J. et al. Glutamine deprivation initiates reversible assembly of mammalian rods and rings. Cell. Mol. Life Sci. 71, 2963–2973 (2014).
22. Martin, E. et al. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. Nature 510, 288–292 (2014).
23. Hofer, A., Steverding, D., Chabes, A., Brun, R. & Thelander, L. Trypanosoma brucei CTP synthetase: a target for the treatment of African sleeping sickness. Proc. Natl. Acad. Sci. USA 98, 6412–6416 (2001).
24. Hindenburg, A.A., Taub, R.N., Grant, S., Chang, G. & Baker, M.A. Effects of pyrimidine antagonists on sialic acid regeneration in HL-60 cells. Cancer Res. 45, 3048–3052 (1985).
25. Kang, G.J. et al. Cyclopentenylcytosine triphosphate. Formation and inhibition of CTP synthetase. J. Biol. Chem. 264, 713–718 (1989).
26. Politi, P.M. et al. Phase I clinical trial of continuous infusion cyclophosphorin triphosphate 1. Cancer Chemother. Pharmacol. 36, 513–523 (1995).
27. Trudel, M., Van Genechten, T. & Meuth, M. Biochemical characterization of the hamster thy mutator gene and its revertants. J. Biol. Chem. 259, 2355–2359 (1984).
28. Rauschel, F.M., Thoden, J.B. & Holden, H.M. Enzymes with molecular tunnels. Acc. Chem. Res. 36, 539–548 (2003).
29. Chang, Y.F., Martin, S.S., Baldwin, E.P. & Carman, G.M. Phosphorylation of human CTP synthetase 1 by protein kinase C: identification of Ser(462) and Thr(455) as major sites of phosphorylation. J. Biol. Chem. 282, 17613–17622 (2007).
30. Choi, M.G. & Carman, G.M. Phosphorylation of human CTP synthetase 1 by protein kinase A: identification of Thr(455) as a major site of phosphorylation. J. Biol. Chem. 282, 5367–5377 (2007).
31. Choi, M.G., Park, T.S. & Carman, G.M. Phosphorylation of Saccharomyces cerevisiae CTP synthetase at Ser424 by protein kinases A and C regulates phosphatidylcholine synthesis by the CDP-choline pathway. J. Biol. Chem. 278, 23610–23616 (2003).
32. Han, G.S. et al. Expression of Human CTP synthetase in Saccharomyces cerevisiae reveals phosphorylation by protein kinase A. J. Biol. Chem. 280, 38328–38336 (2005).
ONLINE METHODS

Purification of ecCTPS. Wild-type ecCTPS was purified as described previously11. The ecCTPS H355A mutant was purified in the same manner, except 10 mM DTT was included throughout the purification.

Purification of hCTPS1. hCTPS1 was expressed in Saccharomyces cerevisiae strain GKY55, as described by Han et al., 2005 (ref. 32). This strain lacks the endogenous S. cerevisiae CTPS genes URA7 and URA8, and contains plasmid pDO105-hCTPS1, which directs expression of His6-tagged (C-terminal) hCTPS1 from the ADH1 promoter. GKY55 cells were grown in 4× YPD media and harvested by freezing cell pellets in liquid nitrogen. Cell pellets were ground to a powder while frozen, and 15 g of cell powder was resuspended in 100 mL of lysis buffer (50 mM Tris-HCl, 200 mM NaCl, 0.3 M sucrose, 20 mM imidazole, 0.5 mM PMSF, pH 8.0). Lysates were clarified by centrifugation at 14,000 rpm for 40 min at 4 °C in a Thermo Scientific Fibrelite F14-14 × 50 cy rotor. Clarified lysates were applied to a 5 mL HisTrap FF Crude column (GE) on an AKTA Start chromatography system (GE), and the column was washed with 30 column volumes (CV) wash buffer (20 mM Tris-HCl, 0.5 M NaCl, 45 mM imidazole, 10% glycerol, pH 7.9). Protein was eluted as 1 mL fractions with 5 CV elution buffer (20 mM Tris-HCl, 0.5 M NaCl, 250 mM imidazole, 10% glycerol, pH 7.9). Fractions containing hCTPS1 were pooled and dialyzed into storage buffer (20 mM Tris-HCl, 0.5 M NaCl, 10% glycerol, 7 mM β-mercaptoethanol, pH 7.9) using Snakeskin 3500 MWCO dialysis tubing (Thermo Scientific). Dialyzed protein was concentrated 6-fold by centrifugation in a 3 kDa cut-off centrifugal filter unit (Millipore). hCTPS1 prepared for cryo-EM was purified in the same manner except protein was concentrated before dialysis and then dialyzed into 20 mM Tris-HCl 50 mM NaCl 7 mM β-mercaptoethanol, pH 7.9. The hCTPS1−H355A mutant was generated by site-directed mutagenesis of plasmid pDO105-hCTPS1 and purified in the same manner as WT hCTPS1.

Site-directed mutagenesis. Site-directed mutagenesis of the E. coli and human CTPS expression constructs was performed using the QuickChange (Agilent Technologies) and Q5 (NEB) systems, respectively.

CTPS activity assays. CTPS activity was determined at 37 °C by measuring ΔA340nm over time using a NanoDrop 2000c spectrophotometer (Thermo Scientific). Absolute CTPS production was calculated using the change in extinction coefficient between UTP and CTP at 291 nm (1338 M−1 cm−1)33. For ecCTPS, assays were performed with 0.5–2.5 µM ecCTPS or ecCTPS C268A in a standard reaction buffer containing 50 mM Na-HEPES (pH 8.0) and 10 mM MgCl2, with or without 10 mM DTT as appropriate. Final substrate concentrations were 0.6 mM UTP, 1.5 mM AMP−PNP, 0.2 mM GTP, and 10 mM glutamine or ammonia. Assays were carried out using the “annealing” method described by Habrian, et al.34; ecCTPS was incubated at 21 °C for 3 min and then combined with pre-warmed (37 °C) nucleotides. The enzyme-nucleotide mix was then incubated further for 4 min at 37 °C, after which pre-warmed glutamine or ammonia was added. The complete reaction mixture was then immediately transferred to a pre-warmed UV cuvette and ΔA340nm over time measured at 37 °C. For hCTPS1, assays were performed with 8 µM hCTPS1 in a standard reaction buffer containing 20 mM Tris-HCl (pH 7.9) and 10 mM MgCl2, with final substrate concentrations of 2 mM UTP, 2 mM ATP, 0.2 mM GTP, and 10 mM glutamine. The annealing method described above was also used for hCTPS1 activity assays, except the enzyme-nucleotide mix was incubated for 1 h at 37 °C to allow for hCTPS1 polymerization.

Negative-stain electron microscopy. Samples for negative-stain EM were prepared by applying CTPS to carbon-coated grids and staining with 0.7% uranyl formate. For ecCTPS C268A, protein was dialyzed overnight into 50 mM Na-HEPES (pH 8.0) with or without 10 mM DTT before being coated onto grids at a final concentration of 2.5 µM. For hCTPS1 and hCTPS1−H355A, samples were prepared with 2.5 µM protein in 20 mM Tris-HCl (pH 7.9) and 10 mM MgCl2, supplemented with appropriate nucleotides at the following concentrations: UTP (2 mM), ATP (2 mM), AMP−PNP (2 mM), GTP (0.2 mM), ADP (2 mM), and CTP (2 mM). hCTPS1 was incubated with nucleotides for 1 h at 37 °C before being coated onto grids. For depolymerization experiments, glutamine (10 mM) was added following incubation with nucleotides, and negative-stain samples were prepared 30 and 120 min following glutamine addition. EM was performed on a Tecnai G2 Spirit (FEI co.) operating at 120 kV, and images were acquired at 52,000× magnification on a US4000 4k × 4k CCD camera (Gatan, Inc.).

Cryo-electron microscopy. Samples for cryo-EM were prepared by applying protein to glow-discharged C-Flat holey-carbon grids (Protochips Inc.), blotting with a Vitrobot (FEI co.), and rapidly plunging into liquid ethane. For ecCTPS−SC268A, protein was dialyzed into nonreducing buffer (50 mM Na-HEPES pH 8.0) to promote filament assembly. Samples for cryo-EM were then prepared with 2.5 µM ecCTPS C268A in the absence of nucleotides or following 1 h incubation of filaments with 10 mM MgCl2 in addition to UTP (0.6 mM) and ATP (1.5 mM) or CTP (0.6 mM) and ADP (1.5 mM). hCTPS1 (5 µM) was incubated for 1 h with 10 mM MgCl2, 2 mM UTP, 2 mM ATP, and 0.2 mM GTP before preparing cryo-EM samples. For ecCTPS tetramers, 5 µM ecCTPS was incubated with 10 mM MgCl2, 0.6 mM UTP, 1.5 mM AMP−PNP, 0.2 mM GTP and 10 mM glutamine before preparing cryo-EM samples. Cryo-EM data for the wild-type ecCTPS filament were acquired in a Tecnai Polara operating at 300 kV and recorded on a K2 Summit Direct Detector camera with a total dose of ~34 e−/Å2 with 36 frames per exposure. For all other samples, cryo-EM data was collected on a Tecnai G2 F20 (FEI co.) operating at 200 kV with a K2 Summit Direct Detector camera (Gatan Inc.) with a pixel size of 1.26 Å/pixel. Movies were acquired in counting mode with 36 frames and a total dose of ~45 e−/Å2 (filaments) or ~68 e−/Å2 (ecCTPS tetramers), and with a defocus range between 0.8 and 2.5 µm (filaments) or 1.0 and 4.5 µm (ecCTPS tetramers). LegoIn software35 was used to automate data collection.

Image processing and 3D reconstruction. Movie frames were aligned using DOSEGPU DRIFTCOR36 and CTF parameters were estimated using CTFIND3 (ref. 37). For helical samples, lengths of helices were manually defined using Appion38 manual picker, and overlapping segments were extracted along the length of each helix. Particle stacks were CTF corrected by phase flipping in SPIDER39, using parameters determined by CTFIND3. 3D reconstruction of helices was performed by iterative helical real space reconstruction (HHRSP)40,41 in SPIDER, using hsearch_lorentz to refine helical symmetry parameters. Cylinders were used as starting models, and the D2 point-group symmetry of the CTPS tetramer was enforced. Iterative gold-standard refinement was performed with increasingly smaller angular increments (minimum 1.5°). For single-particle (tetramer) samples, particles were picked automatically using DoG Picker42 and then extracted using Appion. Relion42 was used for CTF correction (using CTFIND3 parameters) and subsequent classification and refinement. We performed reference-free 2D classification and selected 7,700 and 39,000 particles for 3D classification of the hCTPS1−H355A and ecCTPS tetramers, respectively. For 3D classification, initial reference volumes were generated from the homology model of the hCTPS1 tetramer or crystal structure of the product-bound ecCTPS tetramer. Both reference volumes were low-pass filtered to 60 Å, and D2 symmetry was enforced during 3D classification. Following 3D classification, 4,400 and 6,400 particles were selected for hCTPS1−H355A and ecCTPS, respectively, for gold-standard 3D refinement. Again, reference volumes were low-pass filtered to 60 Å and D2 symmetry was enforced during 3D refinement. The gold-standard FSC = 0.143 criterion was used for estimating resolution. Raw volumes were amplitude corrected and low and high-pass filtered using SPIDER. Details of each 3D reconstruction are summarized in Table 1.

Building and fitting of atomic models. An ecCTPS monomer (PDB 2AD5) was initially fit as a single rigid body into the cryo-EM structure of the filament. For the human filament, an initial homology model of the full-length hCTPS monomer was generated from the crystal structures of the individual domains (PDB 2VO1 and 2VKT) aligned to ecCTPS (PDB 2AD5) using MODELLER45 then fit to cryo-EM density as three rigid bodies: GAT domain, linker domain, and ALase domain. For both structures, atomic models were refined with the Rosetta Relax application, using the cryo-EM density as a constraint. Adjacent protomers within the tetramer and at filament contact sites were included during the Relax procedure to allow for refinement at subunit interfaces.

X-ray structure of an alternate CTP-inhibited ecCTPS conformation. N-terminal His-tagged ecCTPS mutant C268A was expressed and purified using metal chelate chromatography as previously described34; ecCTPS(C268A) had identical kcat.
and CTP inhibition as wild type. Protein for crystallization was stored in 10 mM Tris–Cl, 0.5 mM TCEP, pH 8.0 at −80 °C at 10–15 mg/mL. P2₁,2,2 crystals iso-
morphous to the published apo- and CTP/ADP-ligated structures (PDB 1S1M
and 2AD5) were grown using vapor diffusion from 7.5 mg/mol ecCTPS-C268A,
0.8–1.2 M ammonium sulfate, 0.1 M Tris-Cl, pH 8.0 as previously described14
with drops supplemented with 5 mM CTP, 5 mM magnesium sulfate and 10 mM
adenosine. For crystallization, crystals were briefly rinsed in 1:1 mixture of 50% MPD: mother liquor (25% MPD final), wicked and flash cooled in liquid nitro-
gen. Reflection data were acquired at Stanford Synchrotron Radiation Lightsource Beamline 1-5 at 100 K at a wavelength of 0.979610 Å and 1° oscillation/image,
using an ADSC Quantum 315 detector. 88 frames were processed using DENZO/
Scalepack (HKL2000 package). Phases were derived from the water-free published
CTP and ADP complex structure (PDB 2AD5). Maximum-likelihood structure
factor refinement was carried out on nonhydrogen atoms using Refmac 5.6 with
Babinet scaling with an anisotropic B-factor correction (BSOL fixed at 125.00
and final anisotropic scaling parameters B_{11} = −1.82, B_{22} = 2.83, B_{33} = −1.01,
B_{12} = 0.00, B_{13} = 0.00, B_{23} = 0.00). Quaternary changes were accounted for by
initial rigid-body refinement for individual monomers and then for three separate
domains of each monomer (1–266, 267–287, 288–544). Cycles of manual model
building with Coot 0.6.2 and combined positional and B-factor refinement led to
the final model. The distribution of favored, allowed, and outlier Ramachandran
angles were 94, 5 and 1%, respectively (51st percentile for structures at this resolu-
tion). The data collection and final model statistics are provided in Table 2.

Sequence comparison. CTPS sequences were obtained from BLAST searches47,
and multiple sequence alignments of several hundred sequences were performed
with MAFFT48.

Data availability. Coordinates and structure factors for the CTP-inhibited
ecCTPS crystal structure have been deposited in the Protein Data Bank under
accession code PDB 5TKV. EM structures and associated atomic models have been deposited in the Electron Microscopy Data Bank and Protein Data
Bank with the following accession codes: ecCTPS filament (EMD-8504; PDB
5U3C), hCTPS1 filament (EMD-8474; PDB 5U03), hCTPS1-H355A tetramer
(EMD-8476), ecCTPS tetramer (EMD-8475; PDB 5U05), ecCTPS-CC (apo
EMD-8490), ecCTPS-CC (substrates) (EMD-8491), ecCTPS-CC (products
EMD-8513; PDB 5U6R). Other data supporting this study are available from
the corresponding authors upon reasonable request.

33. Long, C.W. & Pardee, A.B. Cytidine triphosphate synthetase of Escherichia coli
B. I. Purification and kinetics. J. Biol. Chem. 242, 4715–4721 (1967).
34. Habrian, C. et al. Inhibition of Escherichia coli CTP synthetase by NADH and other
nicotinamides and their mutual interactions with CTP and GTP. Biochemistry 55,
5554–5565 (2016).
35. Suloway, C. et al. Automated molecular microscopy: the new Leginon system.
J. Struct. Biol. 151, 41–60 (2005).
36. Li, X. et al. Electron counting and beam-induced motion correction enable near-
atomic-resolution single-particle cryo-EM. Nat. Methods 10, 584–590 (2013).
37. Mindell, J.A. & Grigorieff, N. Accurate determination of local defocus and specimen
tilt in electron microscopy. J. Struct. Biol. 151, 334–347 (2003).
38. Lander, G.C. et al. Appion: an integrated, database-driven pipeline to facilitate EM
image processing. J. Struct. Biol. 166, 95–102 (2009).
39. Frank, J. et al. SPIDER and WEB: processing and visualization of images in 3D
electron microscopy and related fields. J. Struct. Biol. 116, 190–199 (1996).
40. Egelman, E.H. The iterative helical real space reconstruction method: surmounting
the problems posed by real polymers. J. Struct. Biol. 157, 83–94 (2007).
41. Sachse, C. et al. High-resolution electron microscopy of helical specimens: a fresh
look at tobacco mosaic virus. J. Mol. Biol. 371, 812–833 (2007).
42. Egelman, E.H. A robust algorithm for the reconstruction of helical filaments using
single-particle methods. Ultramicroscopy 85, 225–234 (2000).
43. Voss, N.R., Yoshioka, C.K., Radermacher, M., Potter, C.S. & Carragher, B. DoG
Packer and TiltPicker: software tools to facilitate particle selection in single particle
electron microscopy. J. Struct. Biol. 166, 205–213 (2009).
44. Scheres, S.H. RELION: implementation of a Bayesian approach to cryo-EM structure
determination. J. Struct. Biol. 180, 519–530 (2012).
45. Webb, B. & Salì, A. Comparative protein structure modeling using MODELLER.
Curr. Protoc. Bioinformatics 54, 5.6.1–5.6.37 (2016).
46. DiMaio, F. et al. Atomic-accuracy models from 4.5-Å cryo-electron microscopy data
with density-guided iterative local refinement. Nat. Methods 12, 361–365
(2015).
47. Johnson, M. et al. NCBI BLAST: a better web interface. Nucleic Acids Res. 36,
W5–W9 (2008).
48. Katoh, K. & Standley, D.M. MAFFT multiple sequence alignment software version
7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780
(2013).
Supplementary Figure 1

Cryo-EM reconstruction of ecCTPS.

a, Cryo-electron micrograph of ecCTPS filaments. b, The resolution of the structure is 4.6 Å by gold-standard FSC. c, Local resolution map of one ecCTPS tetramer in the filament. d, A central slice through the unfiltered ecCTPS filament reconstruction shows less well-defined features in the GAT domains (yellow arrow) than in the AL domains (white arrowhead).
Details of structural rearrangements at the CTP binding site that create new intersubunit contacts and drive the conformational switch to the filament-forming conformation.

**a**, The alternative ecCTPS-CTP complex structure (5TKV) is shown in yellow (subunit A), red (subunit B) and green (subunit A', two-fold symmetry related to A, symmop 4). The previously published CTP complex (2AD5\textsuperscript{16}) is shown in gray, after superposition of the A subunits (residues 1-266, 0.42 Å C\textsubscript{α} rmsd after superposition). Side chains that have altered interactions or conformations relative to 2AD5 are displayed. Hydrogen bonds unique to 5TKV are shown as cyan dotted lines, and heavy atom distances are indicated. The 2AD5 CTP is depicted using narrow sticks. In 5TKV, an octahedral Mg\textsuperscript{2+} ion coordinates the β- and γ-phosphate oxygens and 3 water molecules. Relative to the A subunit, helices α5(A') and α7(A) C\textsubscript{α} atoms shifted 1.9 Å and 1.1 Å respectively. F227 and N229 side chains changed rotamers, with the F227 phenyl ring packed against the CTP C5 atom, while N229 formed new inter-subunit hydrogen bonds with R158. These new hydrogen bonds became possible due to a quaternary rearrangement of subunits that was similar to the rearrangement of N-terminal domains in the filament-binding conformer observed by cryoEM. This structure validates the sequence conservation for these residues. Mutations at E155, R158 and position 229 confer pyrimidine drug resistance in CTPSs, presumably by hindering the conformational change required for formation of the filament (bacterial\textsuperscript{49}) or inhibited tetramer (yeast\textsuperscript{50}, mammalian\textsuperscript{51}). Similar intersubunit contacts are observed in tetramer crystal structures of the *M. tuberculosis* CTPS-diUTP complex (PDB 4ZDJ), *T. thermophilus* CTPS-glutamine (PDB 1VCO) and apo (PDB 1VCM) complexes, and the hCTPS1 AL domain (PDB 2VO1).

**b**, E155 and N229 participate in extended hydrogen bond networks in the A' and B subunits. Hydrogen bonds specific to 5TKV are depicted in magenta, while those common to both 2AD5 and 5TKV are colored light blue. R158 and N229 form the center of this network.

49. Wylie, J.L., Wang, L.L., Tipples, G. & McClarty, G. A single point mutation in CTP synthetase of Chlamydia trachomatis confers resistance to cyclopentenyl cytosine. *J Biol Chem* 271, 15393-400 (1996).
50. Ostrander, D.B., O'Brien, D.J., Gorman, J.A. & Carman, G.M. Effect of CTP synthetase regulation by CTP on phospholipid synthesis in Saccharomyces cerevisiae. *J Biol Chem* 273, 18992-9001 (1998).
51. Whelan, J., Phear, G., Yamauchi, M. & Meuth, M. Clustered base substitutions in CTP synthetase conferring drug resistance in Chinese hamster ovary cells. *Nat Genet* 3, 317-22 (1993).
**Supplementary Figure 3**

CTP promotes disassembly of hCTPS1 filaments.

**a.** Adding glutamine to hCTPS1 filaments assembled with UTP, ATP, and GTP causes them to disassemble over time.  
**b.** Adding glutamine to hCTPS1 filaments assembled with UTP, AMPPNP, and GTP does not lead to filament disassembly, suggesting CTP synthesis is required to promote hCTPS1 depolymerization.  
**c.** Directly adding CTP to hCTPS1 filaments assembled with UTP, ATP, and GTP causes filaments to disassemble. Scale bars are 50 nm.
Supplementary Figure 4
Cryo-EM reconstruction of hCTPS1.

**a,** Section from a micrograph of frozen hCTPS1. **b,** FSC curve for the hCTPS1 filament, showing a resolution of 6.1Å. **c,** ResMap local resolution map of the hCTPS1 filament. **d,** Atomic model of the hCTPS1 monomer fit into the hCTPS1 filament cryoEM map, colored by domain. **e,** Individual helices are well-defined in the hCTPS1 filament cryoEM map. **f,** Comparison of the ecCTPS and hCTPS1 filament contact sites. hCTPS1 filaments lack the linker-linker contact site, and have a completely rearranged GAT contact site. **g,** Poorly-resolved C-terminal contact site (orange) in the hCTPS1 filament.
Supplementary Figure 5

Mutation of a conserved histidine in the eukaryotic GAT-domain insert abolishes hCTPS1 polymerization without disrupting tetramer formation.

a, CTPS sequence alignments, showing the appearance of the GAT domain helical insert early in eukaryotic evolution. The conserved histidine (H355 in humans) is shown in red. b, hCTPS1-H355A does not polymerize in the presence of UTP, ATP, and GTP. c, Negative stain reconstruction of the hCTPS1-H355A tetramer in the presence of substrates (grey), fit with an atomic model of hCTPS1 in the filament conformation (colored by protomer). Scale bar is 50 nm.