Cytoplasmic poly(A) polymerases mediate cellular responses to S phase arrest

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The S–M checkpoint delays mitosis until DNA replication is complete; cells defective in this checkpoint lose viability when DNA replication is inhibited. This inviability can be suppressed in fission yeast by overexpression of Cid1 or the related protein Cid13. Fission yeast contain six *cid1/cid13*-like genes, whereas budding yeast has just two, *TRF4* and *TRF5*. Trf4 and Trf5 were recently reported to comprise an essential DNA polymerase activity required for the establishment of sister chromatid cohesion. In contrast, we find that Cid1 is not a DNA polymerase but instead uses RNA substrates and has poly(A) polymerase activity. Unlike the previously characterized yeast poly(A) polymerase, which is a nuclear enzyme, Cid1 and Cid13 are constitutively cytoplasmic. Cid1 has a degree of substrate specificity *in vitro*, consistent with the notion that it targets a subset of cytoplasmic mRNAs for polyadenylation *in vivo*, hence increasing their stability and/or efficiency of translation. Preferred Cid1 targets presumably include mRNAs encoding components of the S–M checkpoint, whereas Cid13 targets are likely to be involved in dNTP metabolism. Cytoplasmic polyadenylation is known to be an important regulatory mechanism during early development in animals. Our findings in yeast suggest that this level of gene regulation is of more general significance in eukaryotic cells.

After exposure to hydroxyurea (HU), an inhibitor of ribonucleotide reductase, eukaryotic cells arrest in S phase and do not enter mitosis, owing to the activation of the DNA replication (S–M) checkpoint. In the fission yeast *Schizosaccharomyces pombe*, loss of function of S–M checkpoint genes such as *rad3* does not impair normal growth but results in rapid loss of viability when cells are exposed to HU (1). Rad3 is a large protein kinase related to the vertebrate proteins ATR and ATM that, like Rad3, are required for S–M and DNA damage checkpoint responses (2). These checkpoints arrest the cell cycle to allow time for repair of damaged DNA or completion of DNA replication.

During DNA replication, cohesion is established between newly replicated sister chromatids, which are held together by the multiprotein complex cohesin (3). Maintenance of sister chromatid cohesion from S phase through G_2 and until the onset of anaphase is necessary for accurate chromosome segregation. Cohesion is also important for efficient DNA repair, as it ensures that the undamaged sister chromatid is positioned to serve as a template for repair by homologous recombination. The mechanism by which cohesion is established during S phase is unknown. Recent data suggest that Trf4 and Trf5, two closely related proteins in *Saccharomyces cerevisiae*, act as a DNA polymerase to couple cohesion to replication, possibly directly by replicating sites of cohesion (4). The *S. pombe* genome contains six *TRF4/5* related genes: *cid1*, *cid11*, *cid12*, *cid13*, *cid14*, and *cid16*.

S. pombe cid1 is required for S–M checkpoint integrity when DNA polymerase (Pol) δ or ε is inhibited (5). Overexpression of *cid1* confers resistance to a combination of HU and caffeine, which inhibits the S–M checkpoint (6). Cid1–Cid16, Trf4, and Trf5 are members of the Pol β family of nucleotidyl transferases (7). It initially seemed plausible that *cid1* might also encode a

Pol, possibly related to the establishment of cohesion. However, alternative biochemical functions for Cid1 could not be ruled out. Other members of the Pol β superfamily are known to transfer nucleotidyl residues to proteins, RNAs, or antibiotics (7), and Cid1 lacks analogues of the thumb and finger domains of Pol β that wrap around the DNA substrate (8).

Here, we show that Cid1 has poly(A) polymerase activity and describe the isolation of *cid13*⁺ as a suppressor of the HU sensitivity of a *rad3* mutant. We show that Cid1 and Cid13 constitutively localize to the cytoplasm, suggesting that their targets are also cytoplasmic.

Materials and Methods

Fission Yeast Strains and Methods. Conditions for growth, maintenance, and genetic manipulation of fission yeast were as described (9). Strains *cid13Δ* (*cid13::ura4* or *cid13::LEU2*), *cid13:Myc*, *cid1:HA*, and *cid1:GFP* were constructed in this study. All other strains have been described (1, 5, 10). For gene disruption and tagging, the one-step targeted recombination method was used (11), following PCR-mediated generation of linear DNA fragments marked with *ura4*⁺, *LEU2*, or *kanMX*.

The genomic library screen for suppressors of *rad3*ᵗˢ HU sensitivity has been described (10). The *cid13* insert contained a single ORF originally designated SPAC821.04c and now named *cid13*, according to the nomenclature established for *cid1*, *cid11*, and *cid12* (5). Subclones were capable of suppressing the HU sensitivity of *rad3*ᵗˢ cells only if they contained the whole *cid13* ORF. Multiple sequence alignments were generated by using CLUSTALX software (National Center for Biotechnology Information) and the following parameters: gap opening, 10.0; gap extension, 0.05.

S. pombe protein extract preparation, immunoblotting, anti-Cds1 immunoprecipitations, and Cds1 kinase assays were performed as described (12). Immunostaining with anti-Myc antibody 9E10 was performed by using the general method described (13).

Purification of Recombinant Cid1. cDNAs encoding Cid1 or Cid1DADA (Cid1 with aspartate residues 101 and 103 replaced by alanine) were cloned into pET30a (Invitrogen), transformed into *Escherichia coli* BL21, and expression of the recombinant protein was induced by using 0.3 mM isopropyl β-D-thiogalactoside for 2 h at 37°C. Cells were lysed by sonication on ice in 20 mM Tris, pH 8/20 mM imidazole/500 mM NaCl/8% glycerol with protease inhibitors (Complete, Roche). The lysate was cleared by centrifugation and filtration through a Millex-HA 0.45-μm filter (Millipore), before being loaded onto an Ni²⁺

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charged chromatography column (POROS MC20) using a BioCAD workstation. The column was washed with 20 mM imidazole, and His-6Cid1/Cid1DADA was eluted with an imidazole gradient (50–500 mM over 10 ml). The majority of the protein eluted in a narrow peak across four 1-ml fractions. The identity of each protein was confirmed by using anti-His-6 immunoblotting and N-terminal sequencing.

ATPase and Pol Assays. ATPase activity was determined by thin-layer chromatography following release of ^{32}P from $[\gamma-^{32}P]ATP$, as described (14). Primers for Pol assays were 5′ labeled by using T4 polynucleotide kinase (Roche) and $[\gamma-^{32}P]ATP$. A 2-fold excess of the oligonucleotide template (0.02 pmol) was annealed with one equivalent of the oligonucleotide primer (0.01 pmol) in 30 μl of 1 × Expand PCR buffer (Roche) by slow cooling from 96°C to room temperature. The DNA was then ethanol precipitated and resuspended in 20 μl of TE buffer. Pol reactions (10 μl) contained 25 mM potassium phosphate, pH 7/5 mM MgCl$_2$/5 mM DTT/100 μg/ml BSA/10% glycerol/100 μM dNTPs/10 nM labeled annealed primer/template/200 ng of protein. Reactions were incubated at 30°C for 30 min and stopped by the addition of two volumes of 50 mM EDTA/1% SDS. DNA was ethanol precipitated, resuspended in 90% for-mamide loading buffer, subjected to 8 M urea/15% PAGE, and visualized by using a Storm phosphorimager (Molecular Dynamics).

RNA Polymerase Assays. Template-independent polyadenylation assays were carried out as described (15). Reactions with radio-labeled cordycepin 5′-triphosphate and extension of radiola-beled RNA oligonucleotides using NTPs were measured under the same conditions. Reactions (20 μl) contained 20 mM Tris·HCl, pH 7.5, 50 mM KCl, 0.7 mM MnCl$_2$, 15 mM MgCl$_2$, 0.2 mM EDTA, 100 μg/ml acetylated BSA, 10% glycerol, 5 μl of RNA (1.5 μg), and 1–5 μl of poly(A) polymerase (United States Biochemical, Amersham Pharmacia), Cid1, or Cid1DADA. Reaction products were separated by 7M urea/6% PAGE in 1 × TBE.

Results

Cid1 Is a Poly(A) Polymerase. The Pol β superfamily to which Cid1 belongs is characterized by the sequence motif $hG[G/S]X_{9-13}Dh[D/E]h$ (where X = any amino acid, h = hydrophobic residue), which is a reliable predictor of nucleotidyl transferase activity (7). We therefore decided to characterize the nucleotidyl transferase activity of recombinant Cid1 protein *in vitro*.

Hexahistidine-tagged Cid1 was expressed in *E. coli* and purified by nickel chelate chromatography. We previously generated a cDNA (Cid1DADA) encoding Cid1 with aspartate residues 101 and 103 replaced by alanine (5). The equivalent residues are known to be essential for catalysis in Pol β and poly(A) polymerase (8, 16). Recombinant Cid1DADA was therefore purified to provide a negative control. On SDS/PAGE, both proteins migrated at ≈55 kDa, and silver staining showed that they had been purified to apparent homogeneity (Fig. 1A). As a putative nucleotidyl transferase, Cid1 might be expected to possess ATPase activity, even in the absence of a nucleotidyl recipient substrate. Cid1 hydrolyzed ATP *in vitro*, and whereas this ATPase activity was relatively modest, recombinant Cid1DADA under the same conditions exhibited ATPase activity close to background (Fig. 1B). ATP hydrolysis by Cid1 was maximal in the presence of 2.5 mM Mg^{2+}, although activity was also detected in the presence of other divalent metal ions (data not shown).

To address the possibility that Cid1 might have Pol activity, we assayed recombinant Cid1 and Cid1DADA by using the method previously used to demonstrate that hexahistidine-tagged re-
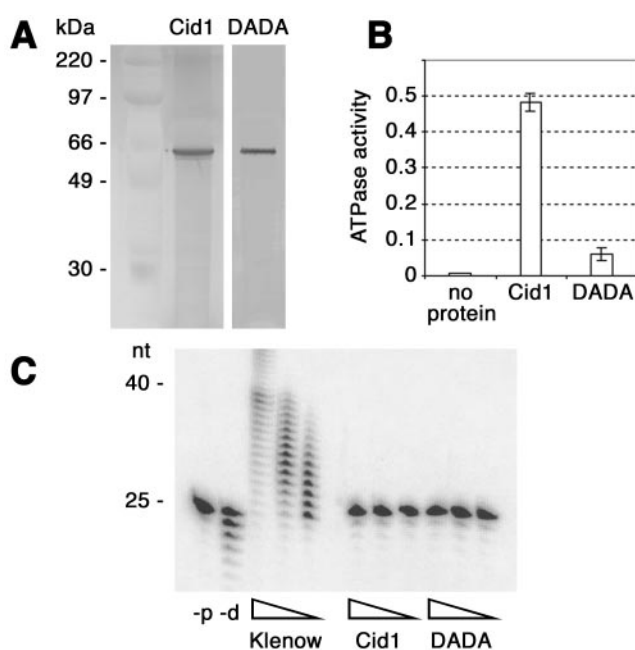

Fig. 1. Recombinant Cid1 is an ATPase but lacks Pol activity. (A) Purified recombinant Cid1 and Cid1DADA were separated by SDS/PAGE and silver-stained. The sizes of the molecular mass markers in the lane next to Cid1 are indicated on the left. (B) ATPase activities (μmol min^{-1}) in the presence of no added protein or 250 ng of recombinant Cid1 or Cid1DADA, as indicated; the average of three independent determinations is shown graphically (error bars represent one standard deviation). (C) *In vitro* Pol assays were performed by using a dT$_{25}$/dA$_{40}$ substrate and 5-fold serial dilutions of *E. coli* PolI large fragment (Klenow), Cid1, or Cid1DADA as indicated. Control reactions included either no added protein (−p) or Klenow enzyme in the absence of added dNTPs (−d). The sizes of reaction products in nucleotides are indicated on the left.

combinant Trf4 (Pol σ) is a Pol (4). The large fragment of *E. coli* Pol I, used as a positive control in these assays, was able to extend the dT$_{25}$ primer efficiently. In contrast, neither recombinant Cid1 nor Cid1DADA exhibited any detectable Pol activity under these conditions (Fig. 1C).

The Pol β superfamily includes the RNA nucleotidyl trans-ferase poly(A) polymerase, which, at the primary sequence level, is more closely related to Cid1 than is Pol β. To test whether Cid1 has poly(A) polymerase activity *in vitro*, we incubated recombinant Cid1 with a ^{32}P-labeled synthetic RNA substrate (A$_{15}$) in the presence of ATP. Cid1, but not Cid1DADA, extended the ^{32}P-A$_{15}$ substrate in a dose-dependent manner to generate a distribution of products qualitatively similar to that generated by *S. cerevisiae* poly(A) polymerase (Fig. 2A). Cid1 incorporated ≈0.46 nmol of AMP/mg every 10 min under the reaction conditions used, a specific activity ≈5% of that of the commer-cially sourced *S. cerevisiae* poly(A) polymerase. Previously char-acterized poly(A) polymerases have a high degree of specificity for ATP in comparison with other nucleotide triphosphates. In the *in vitro* poly(A) polymerase assay, Cid1 was able to use either ATP or UTP as the donor for processive nucleotidyl transfer, but with GTP, CTP, or dATP, only one to three residues were incorporated (Fig. 2B). The synthesis of poly(U) tracts by Cid1 *in vitro* indicates that Cid1 does not share the high selectivity for ATP that is characteristic of nuclear poly(A) polymerases. However, in the ATPase assay, Cid1 preferentially hydrolyzed ATP in the presence of competing NTPs (Fig. 2C), indicating that *in vivo*, where ATP is likely to be at least 5-fold more abundant than UTP, Cid1 may be sufficiently selective for ATP to allow it to function primarily or solely as a poly(A) polymer-
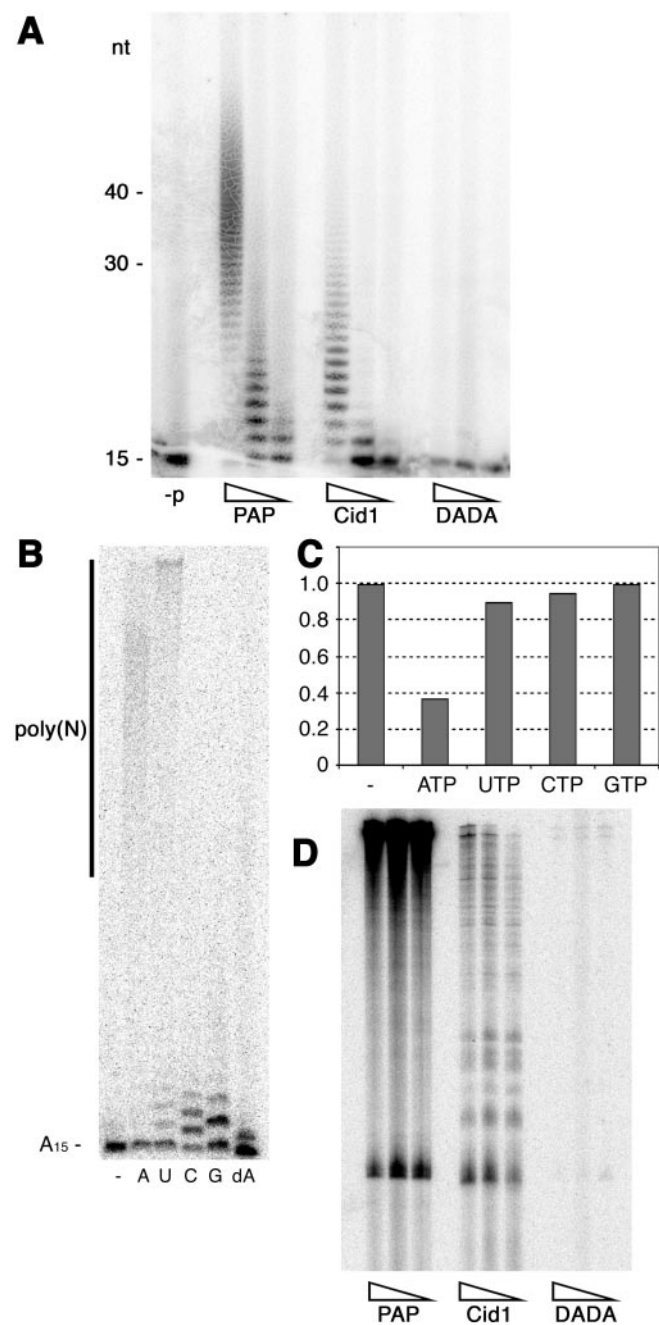
Fig. 2. Cid1 is a poly(A) polymerase. (*A*) *In vitro* poly(A) polymerase assays were performed by using 50, 5, and 0.5 ng of *S. cerevisiae* poly(A) polymerase (PAP), and 200, 40, and 8 ng of Cid1 or Cid1DADA, as indicated, and a radiolabeled A$_{15}$ RNA substrate for 10 min at 30°C. A control reaction included no added protein (−p). The sizes of reaction products in nucleotides are indicated on the left. (*B*) Poly(A) polymerase assays were performed for 30 min at 30°C by using 400 ng of Cid1 and ATP (A), UTP (U), CTP (C), GTP (G), dATP (dA), or no added nucleotide triphosphate (−). (*C*) Cid1 ATPase assays were performed as in Fig. 1*B* (−) or with the addition of a 5-fold molar excess of each of the unlabeled NTPs indicated. (*D*) Poly(A) polymerase assays were performed as in *A*, but using 2-fold serial dilutions of each enzyme, total *S. pombe* RNA, and [α-^{32}P]cordycepin 5′-triphosphate.

ase. Unlike nuclear poly(A) polymerase, Cid1 was not appreciably stimulated by Mn^{2+} (data not shown).

To investigate whether Cid1 might have inherent substrate specificity when presented with a complex mixture of RNA molecules, we incubated total *S. pombe* RNA with Cid1,

Cid1DADA, or *S. cerevisiae* poly(A) polymerase, in the presence of the chain terminating nucleotide [α-^{32}P]cordycepin 5′-triphosphate (3′ deoxy-ATP). In comparison with *S. cerevisiae* poly(A) polymerase, which was able to end-label a continuous smear of RNA species under these conditions, Cid1 appeared to target a subset of *S. pombe* RNAs preferentially (Fig. 2*D*). Cid1 was able to extend synthetic RNA oligonucleotides with different 3′ nucleotides with similar efficiencies (data not shown). This could indicate that Cid1 does not preferentially extend preexisting poly(A) tails *in vivo*; alternatively, specificity for polyadenylated substrates might be conferred by interaction with other factors.

cid13 Suppresses rad3^{ts} HU Sensitivity. We have shown that overexpression of *cid1* partially suppresses the HU sensitivity of *rad3* mutants (5). To identify additional components with related functions, we used an *S. pombe* genomic library to identify multicopy suppressors of the HU sensitivity conferred by partial loss of Rad3 function in a *rad3*^{ts} strain (10). This screen identified the gene *cid13*, as well as those encoding Suc22, the small subunit of ribonucleotide reductase, and Cds1, the checkpoint kinase that acts downstream of Rad3 in the S–M checkpoint. The *cid13* ORF encodes a 65-kDa protein of 579 aa residues with 31% identity to Cid1 (51% similarity) between amino acid residues 56 and 356 (Fig. 3*A*). Protein family (Pfam) domain structure analysis indicated that Cid13 contains a poly(A) polymerase-related domain (PAP/25A core domain) between residues 91 and 232 and a PAP/25A-associated domain between residues 275 and 336. Overexpression of *cid13* (*cid13*^{oe}) partially suppressed the HU and UV sensitivities both of *rad3*^{ts} and of the *rad3*^{kd} (kinase dead) strain, which is defective in checkpoint signaling to the same extent as a *rad3* deletion (Fig. 3 *B–D*). Thus, *cid13*^{oe} may function as a bypass suppressor of *rad3* loss of function. Rad3 activates the downstream protein kinase Cds1 when DNA replication is inhibited. To determine whether *cid13*^{oe} bypasses Rad3 and also bypasses activation of Cds1, we assessed Cds1 kinase activity in *rad3*^{ts} cells containing either the *cid13* plasmid or the empty vector, after incubation in HU at 32°C (Fig. 3*E*). There was no significant difference between the Cds1 activities in these extracts, supporting the notion that Cid13 suppresses the Rad3 defect by a bypass mechanism.

Of all known gene products, Cid13 is most closely related to Cid11 of *S. pombe*. We therefore examined the relationship between *cid13* and *cid11*. Multicopy plasmid expression of a genomic *cid11* clone did not suppress the HU sensitivity of *rad3*^{ts} cells at 32°C (data not shown). Like *cid11*Δ (null) mutants, *cid13*Δ and *cid13*Δ *cid11*Δ double mutants showed no obvious growth or morphology phenotypes (data not shown). However, unlike *cid11*Δ, *cid13*Δ strains were hypersensitive to prolonged exposure to HU (Fig. 3*F*). *cid13*Δ *cid11*Δ double mutants were as sensitive to HU as single *cid13*Δ mutants. None of the single or double mutants exhibited sensitivity to UV or gamma irradiation (data not shown). Significantly, deletion of *cid13* increased the HU sensitivity of *rad3*^{ts} and *rad3*Δ mutants (Fig. 3*F*).

These data suggest that *cid13* acts independently of *rad3* to promote survival after exposure to HU. Several explanations for this are possible. First, *cid13* might control a *rad3*-independent checkpoint response. Alternatively, *cid13* could improve the efficiency of DNA replication or repair processes, or could influence the toxicity of HU either directly or indirectly. We assayed the appearance of unscheduled mitotic ("cut") cells after HU treatment of *cid13*Δ, *rad3*Δ, and *rad3*^{ts} cells (with or without *cid13*^{oe}). No significant effects of *cid13* on the S–M checkpoint were seen in any case (data not shown). Although we cannot exclude a direct role for *cid13* in DNA repair or replication, we did not observe changes in the rate of DNA replication in *cid13*Δ cells by flow cytometry of cells synchronized by
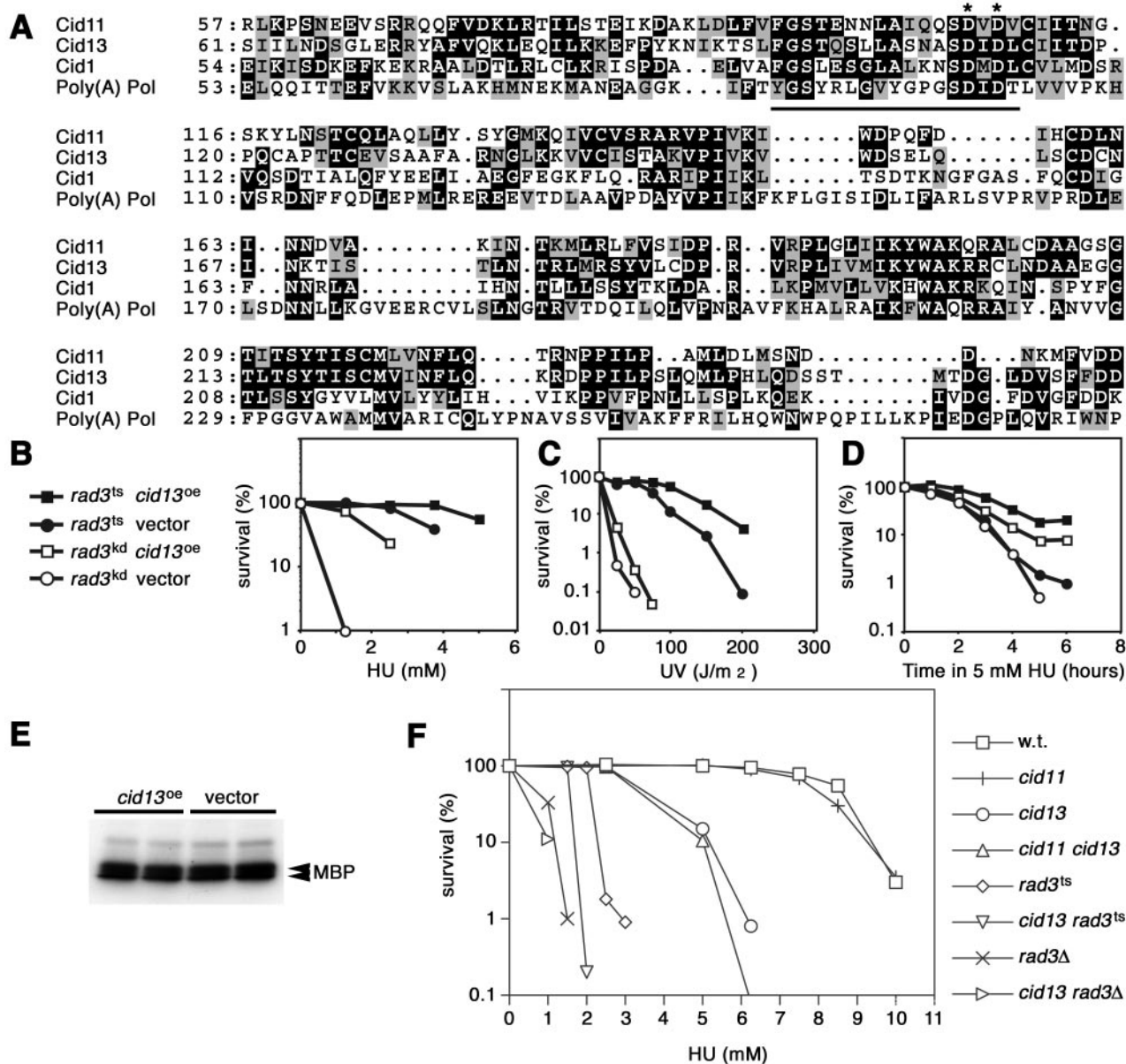
Fig. 3. Cid13 is a Cid1- and poly(A) polymerase-related protein that suppresses the HU sensitivity of *rad3* mutants. (*A*) Alignment of amino acid sequences of the putative catalytic cores of Cid1, Cid11, Cid13, and *S. pombe* poly(A) polymerase. Amino acid residue numbers are indicated to the left of each sequence, conserved residues are highlighted on a black background, and conservative substitutions are shaded. The Pol β superfamily motif is underlined, and the two conserved aspartate residues changed to alanine in the Cid1 DADA mutant are indicated by asterisks. (*B* and *C*) Survival of *rad3*^ts or *rad3*^kd cells transformed with pUR-cid13 (*cid13*^oe) or pUR (vector), as indicated, after plating onto agar containing various doses of HU (*B*) or after UV irradiation on agar plates (*C*). Survival was scored by counting colonies after 4 days of incubation at 32°C. (*D*) Survival of the same strains after short-term exposure to 5 mM HU in liquid culture at 32.5°C. Aliquots of cells taken at the times indicated were scored for viability by plating onto agar lacking HU and incubation at 32°C for 3 days. (*E*) Cells of the *rad3*^ts strain transformed with pUR-cid13 (*cid13*^oe) or pUR (vector), as indicated, were exposed to 5 mM HU at 32°C in duplicate liquid cultures for 3 h and were then assayed for Cds1 kinase activity *in vitro* using myelin basic protein (MBP) as substrate. (*F*) The strains indicated were inoculated onto plates containing various doses of HU, and survival was scored by counting colonies after 4–5 days of incubation at 32°C.

centrifugal elutriation or observe any UV, methyl methane sulphonate, or ionizing radiation sensitivity (data not shown). We infer that *cid13* functions to affect dNTP metabolism in such a way as to maintain dNTP pools.

Cid1 and Cid13 Are Cytoplasmic Proteins. To characterize the physiological roles of Cid1 and Cid13 in more detail, we fused sequences encoding GFP, Myc, or hemagglutinin (HA) epitopes to the 3′ ends of the *cid1* and *cid13* ORFs at their normal chromosomal loci. *cid1:GFP* and *cid1:HA* strains were not sensitive to the combination of HU and caffeine, and *cid13:Myc*

was not sensitive to HU, indicating that the tags do not affect Cid1 or Cid13 function (data not shown). Surprisingly, given the poly(A) polymerase activity of Cid1, Cid1–GFP, Cid1–HA, and Cid13-myc appeared exclusively cytoplasmic in exponentially growing cells (Fig. 4 and data not shown). Because Cid1 is required for checkpoint integrity when Pol δ or ε is inactivated and Cid13 is required for HU resistance, it seemed possible that under these stress conditions, Cid1 or Cid13 might enter the nucleus. Pol δ was inactivated in a temperature-sensitive *cdc27-P11* strain by shifting to the restrictive temperature (36°C) for 4 h. However, the localization of Cid1-GFP was not altered
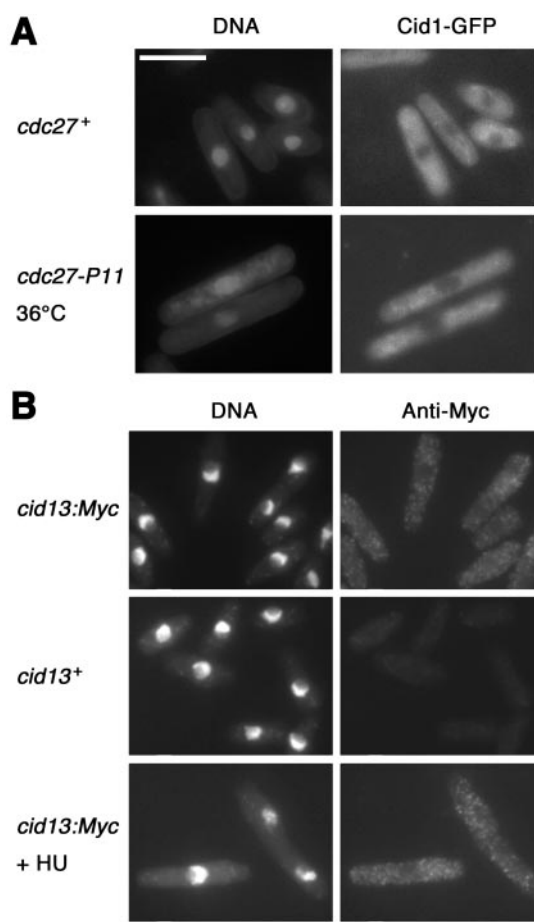
Fig. 4. Cid1 and Cid13 are cytoplasmic proteins. (*A*) Fluorescence micrographs of living *cid1-GFP* cells stained briefly with Hoechst 33258 to reveal DNA (*Left*) and GFP fluorescence (*Right*). (*Upper*) *cid1-GFP* (*cdc27⁺*) cells grown at 32°C. (*Lower*) *cid1-GFP cdc27-P11* cells shifted to 36°C for 4 h. (*B*) Micrographs of fixed wild-type (*cid13⁺*; negative control) and *cid13:Myc* cells, as indicated, processed for anti-Myc immunofluorescence (*Right*) and stained with DAPI to reveal DNA (*Left*). The cells in the lower panels were exposed to 20 mM HU for 3 h before fixation. (Scale bar: 10 μm.)

under these conditions (Fig. 4*A*). Similarly, Cid13 localization was unaffected by incubation in HU (Fig. 4*B*).

Physiological Substrates of Cid1 and Cid13. We identified the *suc22* gene in the same screen through which we isolated *cid13*. We therefore considered the possibility that Cid13 might positively regulate Suc22. Immunoblotting of whole-cell lysates indicated that constitutive Suc22 levels were not significantly altered on deletion of either *cid13* or *cid1* (Fig. 5*A*). Furthermore, no significant change in Suc22 level was seen in wild-type, *cid1Δ*, or *cid13Δ* cells after exposure to HU (Fig. 5*B*). We conclude that the HU sensitivity of *cid13Δ* cells is not attributable to a failure to up-regulate Suc22 protein levels.

Discussion

Cid1 is representative of a class of cytoplasmic proteins with poly(A) polymerase activity. The canonical poly(A) polymerase is responsible for bulk mRNA polyadenylation in the nucleus following the site-specific cleavage of primary PolII transcripts (17). Poly(A) tail length is associated with stability of the mRNA and efficient translation after export to the cytoplasm, as the poly(A) binding protein is an important component of the translation preinitiation complex (18). The cytoplasmic location
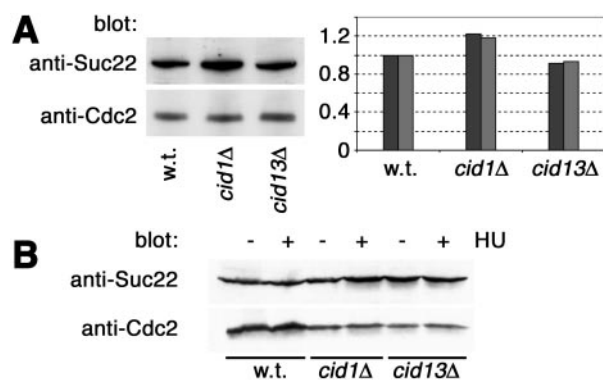

Fig. 5. Expression of Suc22 does not depend on Cid1 or Cid13. (*A*) Suc22 protein levels in whole-cell lysates from wild-type (w.t.), *cid1Δ*, and *cid13Δ* strains were estimated by immunoblotting with a polyclonal anti-Suc22 antibody (*Upper Left*). Protein loading was controlled by reprobing the same filter with an anti-Cdc2 antibody. Suc22 levels in two independent experiments were quantified by densitometry and expressed graphically (*Right*) after normalizing for Cdc2 levels. (*B*) Suc22 and Cdc2 immunoblotting was performed as in *A* from parallel cultures that had been exposed (+) or not (−) to 10 mM HU for 4 h at 30°C.

of Cid1 and Cid13 suggests that their biological functions are distinct from that of nuclear poly(A) polymerase. The distinctive phenotypes of the respective deletion mutants, and the apparent selectivity of Cid1 for particular RNA substrates *in vitro* (Fig. 2*D*), lead us to suggest that Cid1 and Cid13 act to extend the poly(A) tails of distinct subsets of cytoplasmic mRNAs. Poly(A) tail extension would be predicted to increase the levels of the corresponding protein products by promoting mRNA stability and/or translation efficiency. In support of this interpretation, during the preparation of this manuscript Saitoh *et al.* (19) reported that partially purified Cid13 also possessed poly(A) polymerase activity. Other members of the immediate Cid1 family such as Cid11 presumably have significantly different substrate preferences. Our findings also differentiate Cid1 and Cid13 from their closest relative in *S. cerevisiae*, Trf4, which is nuclear and was reported to function as a Pol (4, 20). Multiple Cid1/Cid13-related proteins are found in distantly related eukaryotes including *Caenorhabditis elegans*, plants, and humans. One of these *C. elegans* proteins has recently been identified independently as a cytoplasmic poly(A) polymerase (Liaoteng Wang and Judith Kimble, personal communication). Furthermore, at least one of the human Cid1-like proteins is constitutively cytoplasmic (Soyoung Min and C.J.N., unpublished data). Unlike the canonical poly(A) polymerase, none of the Cid proteins contains a conventional RNA recognition motif. Our *in vitro* data suggest that Cid1 can nonetheless interact productively with RNA in the absence of such a motif. RNA binding partner proteins may act to modulate the efficiency or specificity of such interactions *in vivo*. This might explain the relatively low specific activity of our recombinant Cid1 in comparison with *S. cerevisiae* poly(A) polymerase (Fig. 2*A*). Alternatively, the low specific activity might indicate that only a small proportion of the recombinant protein is properly folded.

Cid1 and Cid13 can each partially suppress the HU sensitivity of *rad3* mutants, but the underlying mechanisms appear to differ. *cid13Δ* cells were hypersensitive to long-term exposure to HU (Fig. 3*F*) but were fully proficient for the S–M checkpoint and not sensitive to short-term HU exposure (data not shown). In contrast, *cid1D* cells were not unusually sensitive to HU, except in the presence of caffeine (5). Suppression of *rad3*ᵗˢ HU sensitivity by overexpression of either *suc22* or *cid13* did not depend on residual *rad3* function (Fig. 3 *B* and *D* and data not shown), and deletion of *cid13* increased the HU sensitivity of

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$rad3^{ts}$ and $rad3\Delta$ cells (Fig. 3F). These observations suggest that *cid13*, like *suc22*, suppresses the HU sensitivity by a bypass mechanism and that *cid13* and *rad3* do not function in the same pathway. In the case of overexpression of Suc22, suppression of HU sensitivity presumably reflects restoration of dNTP pools. Cid13 was recently reported to contribute to the maintenance of steady-state dNTP pools and to promote *suc22* mRNA polyadenylation and stability after exposure to HU (19). Extension of poly(A) tail length would be expected to promote translation of *suc22* mRNA, but our data show that total Suc22 protein levels are not influenced by *cid13* function, either in the presence or absence of HU (Fig. 5). It is a formal possibility that Cid13-mediated polyadenylation in the cytoplasm controls *suc22* mRNA translation in some way that is not reflected in a change in total Suc22 protein level. Alternatively, other targets of Cid13 may be more important for the maintenance of dNTP pools. Cid1 also appears not to regulate Suc22, and the mRNA targets that explain the specific checkpoint defects of the *cid1*Δ strain await identification.

Cytoplasmic polyadenylation of maternal mRNA has been known for some time to be an important regulatory mechanism in early animal development (21). Our demonstration of Cid1 poly(A) polymerase activity, together with the evolutionary conservation of the Cid1 gene family, supports the idea that cytoplasmic polyadenylation might be of more general importance, both in unicellular and in more complex eukaryotes. Cytoplasmic poly(A) polymerase in early *Xenopus* embryos is maximally active in mitosis, when the nuclear enzyme is inhibited by phosphorylation (22). This suggests that the polymerases responsible are fundamentally distinct, but the cytoplasmic activity has not yet been characterized in detail. Signal-induced cytoplasmic polyadenylation has also been described in neurons, although here, too, the polymerase responsible has not yet been identified (23). It will be interesting to determine whether Cid1-related proteins account for any of these cytoplasmic activities and if such polymerases are subject to stimulus-specific regulation.

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