The cleavage/polyadenylation factor I (CF I) is one of four factors required for mRNA 3’ end formation in the yeast *Saccharomyces cerevisiae*. Here we describe the purification of CF I and its separation into two components, CF IA and CF IB. Both components are needed to reconstitute CF I activity in cleavage and poly(A) addition. CF IA consists of a complex of four polypeptides of 76, 70, 50, and 38 kDa, and CF IB is a single 73-kDa polypeptide. The 76- and 38-kDa subunits of CF IA correspond to the previously identified RNA14 and RNA15 proteins. The RNA14 protein, but not the 70- or 50-kDa proteins, coimmunoprecipitates with the RNA15 protein, indicating that RNA14 and RNA15 proteins exist in a tight complex. RNA15 is the only subunit of CF I that can be cross-linked to pre-mRNA.

Messenger RNA 3’ end formation is a required step for mRNA maturation and gene expression. Generation of mRNA 3’ ends occurs by specific endonucleolytic cleavage of longer precursors, followed by addition of poly(A) tails to the upstream fragments. These tails are 50–70 adenosine residues long in yeast and ~300 adenosines long in mammalian cells (1–3). The poly(A) tails regulate the stability and translation efficiency of mRNA. Recent evidence suggests that the polyadenylation process itself is a prerequisite for transport of mRNA from the cell nucleus to the cytoplasm (4).

In mammalian cells, mRNA 3’ end formation requires at least six factors (for reviews, see Refs. 1–3). Cleavage requires the cleavage and polyadenylation specificity factor (CPSF),¹ the cleavage stimulation factor (CstF), two additional factors (mammalian cleavage factors I and II) (5), and mammalian poly(A) polymerase (PAP). The poly(A) addition reaction requires CPSF, PAP, and poly(A)-binding protein II. Most of these factors have been characterized (1–3, 5), and many of their components have been cloned (1–3, 6–10).

In *Saccharomyces cerevisiae*, four separable factors are necessary for mRNA 3’ end formation (11). The cleavage/polyadenylation factor I (CF I) is required for both cleavage and poly(A) addition and hence has an analogous function to mammalian CPSF. CF II and CF I are essential for the cleavage reaction, and the polyadenylation factor I (PF I) and yeast PAP are required in addition to CF I for poly(A) addition (11). Yeast PAP has been purified, and the corresponding gene has been cloned (12–14). Two previously identified genes, RNA14 and RNA15 (15), have been shown to encode components of CF I (16). In addition, the FIP1 gene has been isolated on the basis that its gene product interacts with PAP as a component of PF I (17). FIP1 also associates with the RNA14 protein (RNA14p), suggesting an interaction between CF I, PF I, and PAP (17). Depletion of PAP from yeast extracts with antibodies against PAP decreased cleavage activity (16, 18), which could be restored by addition of CF I (18). These observations also indicate an interaction between CF I and PAP. The interaction of PAP and other polyadenylation factors gives this enzyme specificity and is mediated, at least in part, via the N-terminal end of PAP (19).

In this paper, we describe the purification of yeast CF I and its separation into two functional components, CF IA and CF IB. CF IA activity copurifies with four polypeptides, two of which are RNA14p and RNA15p. These two proteins exist in a tight complex that can be precipitated with antisera to RNA15p. CF IB is a single 73-kDa polypeptide. CF IA and CF IB are required to reconstitute CF I activity in both the cleavage and poly(A) addition reactions, in a manner dependent on processing signals on the precursor RNA. RNA15p is the only subunit of CF I that can be cross-linked to GAL7 pre-mRNA.

**EXPERIMENTAL PROCEDURES**

**Nucleic Acids**—Capped radioactive RNAs used in the processing assays were prepared from the following plasmids by *in vitro* transcription of linearized DNAs as described (11). Full-length precursor containing the *GAL7* poly(A) site and flanking sequences was prepared from pJC-GAL7-10 (11). Preceded wild type *GAL7* RNA, which lacks sequences downstream of the poly(A) site, was prepared from pJC-GAL7-9; and a mutated version of the precleaved RNA, which lacks a (UA)n repeat upstream of the poly(A) site, was prepared from pJC-GAL7-10 (18). All precursor RNAs were purified from 3.3 M urea, 5% acrylamide gels (20); precipitated twice with ethanol; and stored frozen at −20 °C in 50 mM Tris-HCl, pH 7.0.

To express histidine-tagged recombinant RNA14p and RNA15p in *Escherichia coli*, plasmids pET-21b/RNA14 and pET-21b/RNA15 were constructed. The coding regions of RNA14 and RNA15 were amplified from plasmids pLM28 and pFL38-RNA15 (21), respectively, using polymerase chain reaction and were inserted upstream of sequences that code for a C-terminal hexahistidine tag from plasmid pET-21b (Novagen).

**Proteins**—Fractions containing CF II and PF I were obtained by Q-Sepharose chromatography of yeast whole cell extracts as described below. Recombinant PAP was prepared as described (18). Purified bo-
Yeast Whole Cell Extract

Ammonium Sulfate Fractionation

Q-Sepharose

PAP

CF I

Phosphocellulose

CF II

Heparin-Sepharose

Superdex-200

poly(A)-Sepharose

RNA14p (p76)

RNA15p (p38)

p70

p50

S-Sepharose

CF I A

CF I B

Fig. 1. Purification of cleavage/polyadenylation factor I. Shown is a schematic diagram of the methods used to purify CF I, separate it into CF I A and CF I B, and purify each component.

Yeast cell pellets were harvested at 70°C. S. cerevisiae cells (bakers' yeast) were purchased from New England Biolabs Inc. Protein markers used for gel filtration were from Pharmacia Biotech Inc.; equilibrated in buffer C (20 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 10% glycerol, and 0.5 mM DTT) with 50 mM KCl, 1 mM PMSF, 0.6 μM leupeptin, and 2 μM pepstatin A. Elution of proteins from this column and all further steps were carried out without protease inhibitors.

Two ammonium sulfate extracts (2.5 g of protein) were pooled, dialyzed with buffer C to 50 mM KCl, and loaded onto a 130-ml Q-Sepharose Fast Flow column (Pharmacia Biotech Inc.; equilibrated in buffer C + 50 mM KCl) at 140 ml/h. The column was washed with 2 column volumes of this buffer, and proteins were eluted with a gradient (8 column volumes) of 100–500 mM KCl in buffer C, followed by 1 volume of buffer C + 500 mM KCl. PAP elutes from this column with 100 mM KCl, and CF I elutes with 180–275 mM KCl. Fractions containing CF II elute with 300–400 mM KCl, and fractions containing only CF I elute with 400–500 mM KCl. Fractions containing CF I activity were pooled, dialyzed for 2 h against 4 liters of buffer D (20 mM potassium phosphate, pH 6.85, 0.2 mM EDTA, 0.5 mM DTT, and 10% glycerol) supplemented with 100 mM KCl, and applied to a 61-ml phosphocellulose P-11 column (Whatman; equilibrated in the same buffer) at 75 ml/h. After washing with 2 column volumes of this buffer, proteins were eluted with a gradient (3 column volumes) of 100–500 mM KCl in buffer D. Fractions containing CF I (which eluted with 275–285 mM KCl) were pooled, dialyzed against buffer D + 2 liters of buffer C (20 mM Tris-HCl, pH 7.9, and 0.2 mM EDTA) and applied to a prepacked 5-ml heparin-HiTrap column (Pharmacia Biotech Inc.; equilibrated in the same buffer) at 15 ml/h. The column was then washed with 3 volumes of buffer D + 120 mM KCl and then with 5 volumes of buffer D + 240 mM KCl, and CF I was eluted in one step with buffer D + 400 mM KCl.

The material containing CF I (1.67 mg of protein) was then applied to a 110-ml Superdex 200 prep-grade gel filtration column (Pharmacia Biotech Inc.; equilibrated in buffer C + 150 mM KCl, 1.6 × 55 cm) at 36 ml/h. Elution was carried out in this buffer, collecting 1-ml fractions. This column separated CF I into CF I A and CF I B. To determine the elution positions of gel filtration size markers, parallel runs were conducted individually with blue dextran, β-amylase, and BSA, with the same protein concentration, volume, and conditions as the CF I sample.

Fractions containing CF IA activity (see below) were diluted 2-fold in buffer C and applied by gravity flow to a 0.2-ml poly(A)-Sepharose column (Pharmacia Biotech Inc.) equilibrated in buffer C + 75 mM KCl. After washing with 3 column volumes of this buffer, proteins were eluted with 0.6-ml steps of buffer C + 150 mM KCl, 250 mM KCl, 500 mM KCl, or 1 M KCl. CF IA activity eluted with 250 mM KCl.

Fractions with CF IB activity were dialyzed against 1 liter of buffer D + 50 mM KCl for 1.5 h and applied by gravity flow to a 0.6-ml S-Sepharose column (Pharmacia Biotech Inc.) equilibrated in the same buffer. The column was washed with 2 volumes of this buffer and then step-eluted with buffer D containing 100 mM KCl, 250 mM KCl, 500 mM KCl, or 1 M KCl. CF IB activity was found in the 250 and 500 mM KCl fractions. In addition to the purification steps indicated in Fig. 1, CF I was also analyzed by glycerol gradient centrifugation and chromatography on an Affi-Gel blue column.

Glycerol Gradient Centrifugation—0.5 ml of a fraction containing CF I (0.2 mg of protein) from a heparin-Sepharose column was dialyzed against 1 liter of buffer C + 100 mM KCl and 15% glycerol for 90 min.
Purification and Characterization of Yeast CF I

and layered on top of a 15–40% glycerol gradient (11 ml; 14 × 89-mm tube) in buffer C + 100 mM KCl. Sedimentation was performed for 39 h at 4°C at 250,000 × g_{\text{rot}} in a Beckman SW 40 rotor. Fractions of 0.35 ml were manually collected from the top, dialyzed against buffer C + 50 mM KCl, and concentrated for assay. Parallel gradients were done with the following marker proteins: BSA (4.5 S), β-amylose (8.9 S), urease (18.6 S), and carbonic anhydrase (3 S). Marker proteins were located by analysis of fractions on SDS-polyacrylamide gels.

CF I Consists of Two Separable Components—

In an effort to further purify CF I, the heparin-Sepharose fraction was applied to a Superdex 200 gel filtration column. The protein profile after separation on the gel filtration column shows four chromatographic peaks (I–IV) (Fig. 2A). Poly(A) addition assays of individual gel filtration fractions in the presence of PF I and PAP showed no detectable activity (Fig. 2B, lanes 3–13) in comparison with the ammonium sulfate extract or input CF I (lanes 1 and 2). Fractions representing each peak of protein were then pooled, concentrated, and assayed for poly(A) addition activity. Fractions from peaks I–IV showed no detectable activity when assayed alone (Fig. 2C, lanes 4–7). However, poly(A) addition activity was observed when fractions from peak I were combined with those from peak III (Fig. 2C, lane 10) or when fractions from peak II were mixed with those from peak III (lane 13). Combinations of fractions from peaks I and II, peaks III and IV, peaks I and IV, and peaks II and IV showed no activity (lanes 8, 9, 11, and 12). Activity was also observed when fractions from all peaks were mixed (lane 14). These results suggest that CF I was separated into two components, one that resides in a region where peaks I and II overlap (referred to as CF IA) and one that resides in peak III (CF IB).

RESULTS

Purification of Yeast CF I—CF I was originally described as a fraction from Mono Q chromatography of yeast whole cell extract that was required for both the cleavage and poly(A) addition reactions (11). To purify CF I, the preparation of extract was scaled up, and a Q-Sepharose column was used to separate CF I from PAP, CF II, and PF I. CF I was further purified by phoshocellulose and heparin-Sepharose chromatography, yielding an 87-fold purification and a 59% recovery of activity (Table 1). This partially purified CF I participates in the poly(A) addition reaction with precleaved RNA when combined with PAP and PF I (Fig. 2B, lane 2); in the cleavage reaction when combined with CF II (see Fig. 5B, lane 3); and in the complete reaction when combined with CF II, PF I, and PAP (data not shown). This CF I also retains substrate specificity because it can only add poly(A) tails to a precleaved GAL7 RNA that contains a (UA)_{4} repeat (see Fig. 5A, lanes 3 and 9). These results indicate that highly purified CF I retains the properties of the originally described factor (11).

CF I Consists of Two Separable Components—In an effort to further purify CF I, the heparin-Sepharose fraction was applied to a Superdex 200 gel filtration column. The protein profile after separation on the gel filtration column shows four chromatographic peaks (I–IV) (Fig. 2A). Poly(A) addition assays of individual gel filtration fractions in the presence of PF I and PAP showed no detectable activity (Fig. 2B, lanes 3–13) in comparison with the ammonium sulfate extract or input CF I (lanes 1 and 2). Fractions representing each peak of protein were then pooled, concentrated, and assayed for poly(A) addition activity. Fractions from peaks I–IV showed no detectable activity when assayed alone (Fig. 2C, lanes 4–7). However, poly(A) addition activity was observed when fractions from peak I were combined with those from peak III (Fig. 2C, lane 10) or when fractions from peak II were mixed with those from peak III (lane 13). Combinations of fractions from peaks I and II, peaks III and IV, peaks I and IV, and peaks II and IV showed no activity (lanes 8, 9, 11, and 12). Activity was also observed when fractions from all peaks were mixed (lane 14). These results suggest that CF I was separated into two components, one that resides in a region where peaks I and II overlap (referred to as CF IA) and one that resides in peak III (CF IB).
concentrations were measured by the method of Bradford (24) or were estimated from blots stained with Coomassie Brilliant Blue and by separation from CF II, PF I, and PAP. CF IA and CF IB activity was also only measured after separation on the Superdex 200 column. Protein flowed through (50 mM KCl) or eluted with 100 mM KCl showed were required for activity (data not shown).

To determine the fractions with maximal CF IA activity, every third fraction from those spanning peaks I and II (fractions 39–57) was concentrated individually and assayed in the presence of CF IB, PF I, and PAP. This assay localized the maximal CF IA activity to fraction 48, with activity also observed in fractions 45 and 51 (Fig. 2D, lanes 6–8). This places CF IA in the trough between peaks I and II and explains why both peaks showed activity when mixed with CF IB. In a similar manner, fractions spanning peak III (fractions 60–69) were individually concentrated and assayed in the presence of CF IA, PF I, and PAP. This assay localized fractions with maximal CF IB activity to fraction 63, with activity also detected in fraction 60 (Fig. 2D, lanes 11 and 12).

The elution positions of CF IA and CF IB, in comparison with those of marker proteins, indicate that CF IA is a large complex in the 200–300-kDa range, while CF IB is ~100 kDa. Analysis of the gel filtration fractions on a denaturing gel stained with silver shows that CF IA activity cofractionated with several polypeptides whose amount correlates with activity (Fig. 2E, lanes 5–7). In the case of CF IB, such correlation between activity and protein is not possible. These results suggest that the gel filtration column separated CF I into a larger complex (CF IA) and a smaller one (CF IB) that could contain only one polypeptide.

Separation of CF I into two components was also observed by sedimentation in a glycerol gradient or by chromatography on an Affi-Gel blue column. Centrifugation through a 15–40% glycerol gradient separated CF I into two parts, with sedimentation coefficients of 9 and 4.5 S, respectively, by comparison with marker proteins run on parallel gradients. CF I activity was only reconstituted when both components were combined with marker proteins run on parallel gradients. CF I activity present in higher concentration, in agreement with the higher activity observed in the 250 mM KCl fraction compared with the 500 mM KCl fraction (Fig. 3A, lanes 8 and 9). The 250 mM fractions also contain many contaminant polypeptides from the input sample (Fig. 3B, lane 1). These results suggest that CF IB and contaminants both eluted with 250 mM KCl, but some residual CF IB remained in the column and was eluted with 500 mM KCl. To verify that the 500 mM KCl fraction contained only a single polypeptide, an aliquot of this sample was applied to an SDS-polyacrylamide gel, which resolved proteins in the range of 26.6–200 kDa (Fig. 3C).

Purification of CF IA—To further purify CF IA, gel filtration fractions containing CF IA were chromatographed on a poly(A)-Sepharose column. CF IA activity was not detected in the flow-through fractions (75 mM KCl) or in those that eluted with 150 mM KCl, but activity was observed in the middle of three fractions (A2) collected after elution with 250 mM KCl (Fig. 4A, lane 4), with some activity detected in the third fraction (lane 5). Electrophoresis of fraction A2 on a denaturing protein gel stained with silver (Fig. 4B, lane 3) shows that it contains four polypeptides (a–d, with molecular masses of 76, 70, 50, and 38 kDa, respectively), corresponding to four of the polypeptides that cofractionated with CF IA on the gel filtration column. The CF IB polypeptide migrates on SDS-polyacrylamide gels between bands a and b of CF IA (data not shown). The combined mass of the CF IA polypeptides is 240 kDa, assuming that each is represented only once in the complex. The 100-kDa polypeptides seen in fraction A1 peaked in the flow-through and 150 mM KCl fractions.

Specificity of the Reconstituted Poly(A) Addition Reaction—The ammonium sulfate extract cannot add poly(A) to a precleaved substrate that lacks a (UA)_n repeat known to be required for processing of GAL7 mRNA (11). An example of this is shown in Fig. 5A. The extract can polyadenylate the wild type RNA, which contains the (UA)_n repeat, but cannot add poly(A) to the mutant RNA (Fig. 5A, lanes 2 and 8). The same is observed with CF I in the presence of PF I and PAP (Fig. 5A, lanes 3 and 9). No polyadenylation is observed without CF I with either substrate (Fig. 5A, lanes 4 and 10). The specificity of the poly(A) addition reaction with regard to the substrate containing the (UA)_n repeat is also observed with the separated CF IA and CF IB from the gel filtration column (Fig. 5A, lanes 5 and 11) as well as with CF IA and the CF IB from the S-Sepharose column (lanes 6 and 12). These results indicate that the substrate specificity has not been lost after separation and purification of CF I.

**Table I**

| Step                 | Protein | Activity | Specific activity | Yield | Purification |
|----------------------|---------|----------|------------------|-------|--------------|
|                      | mg      | units    | units/mg         | %     | -fold        |
| Crude extract        | 15540   | 2528     | 103,500          | 40.94 |              |
| Ammonium sulfate     | 368     | 4240     | 11.52            | 100   |              |
| fractionation CF I   | 12.45   | 3000     | 241              | 70.8  | 20.9         |
| Q-Sepharose          | 2.50    | 2500     | 1000             | 59.0  | 86.8         |
| CF IA                | 0.18    | 400      | 1111             | 9.43  | 96.4         |
| Superdex 200         | 0.005   | 12       | 2400             | 0.3   | 208.3        |
| Poly(A)-Sepharose    | 0.15    | 300      | 2000             | 7.08  | 173.6        |
| CF IB                | 0.011   | 44       | 4000             | 1     | 347.2        |

TABLE I

Purification of CF I from yeast

The purification from 440 g of yeast cells was as described under “Experimental Procedures.” Quantitation of CF I activity was only possible after separation from CF II, PF I, and PAP. CF IA and CF IB activity was also only measured after separation on the Superdex 200 column. Protein concentrations were measured by the method of Bradford (24) or were estimated from blots stained with Coomassie Brilliant Blue and by comparison with BSA standards.
**CF IA and CF IB Are Both Required for Cleavage Activity**—The results shown thus far indicate that both CF IA and CF IB participate in poly(A) addition. To see if the cleavage reaction requires both components, cleavage assays were done with each component individually and in combination, in the presence of CF II and full-length GAL7 RNA. Under the assay conditions, the ammonium sulfate extract adds poly(A) to all of the cleaved RNA since the upstream cleavage product cannot be detected (Fig. 5B, lane 2). The downstream cleavage product is rarely detectable with the extract, presumably because of degradation. When the assay contains a mixture of CF I and partially purified CF II, the two cleavage products are clearly visible (Fig. 5B, lane 3). No activity is observed if CF I is not added to the reaction (Fig. 5B, lane 4) or if the reaction contains either CF IA or CF IB alone (lanes 5 and 6). Cleavage activity is detected, however, if CF IA and CF IB are combined in the reaction (Fig. 5B, lane 7). The recovered cleavage activity after CF I separation by gel filtration is always lower than the poly(A) addition activity. Inclusion of other fractions from the gel filtration column in the assay did not stimulate the cleavage reaction. If purified CF IB (S-Sepharose 500 mM KCl fraction) is used, very little cleavage activity is recovered (Fig. 5B, lane 8). This could reflect the lower amount of CF IB in this fraction, which also results in lower poly(A) addition activity (Fig. 5A, compare lane 5 and 6), or loss of a component required for cleavage in the S-Sepharose column.

**CF I Contains Both RNA14p and RNA15p—**Minvielle-Sebastia et al. (16) reported that RNA14p and RNA15p are both components of CF I. Protein bands a and d present in CF IA (Fig. 4B, lane 3) have molecular masses that correspond to those of RNA14p and RNA15p, respectively. To determine if these proteins are present in CF IA or CF IB fractions, antibodies raised against recombinant RNA14 and RNA15 proteins were tested for immunoreactivity against blots containing CF I, CF IA, and CF IB, and recombinant proteins. On immunoblots as well as on silver-stained gels, RNA14H6p always appears smaller than yeast RNA14p (Fig. 6A, lanes 2 and 3). The smaller RNA14H6p may have also lost the His6 tag, which might explain the inability of this protein to bind Nif2+ -imidodiacetic acid under denaturing or nondenaturing conditions. RNA15H6p always appears on immunoblots and silver-stained gels as larger than yeast RNA15p (Fig. 6B, lanes 12 and 13) due to the six histidines and two spacer amino acids at the C terminus.

**CF I from the heparin-Sepharose step contains both RNA14p and RNA15p** (Fig. 6, lanes 3 and 12, respectively). These two proteins participate in poly(A) addition and the elution positions of standards. B. poly(A) addition assay of individual Superdex 200 fractions as described under “Experimental Procedures.” The reactions contained 1 μl of the ammonium sulfate extract (lane 1), 1 μl of CF I from the heparin-Sepharose step (HS; lane 2), or 3 μl of Superdex 200 fractions whose numbers are indicated at the top (lanes 3–13). The positions of the poly(A)− and precleaved RNAs on the gel are given on the right. C. poly(A) addition assay of pools of fractions representing each chromatographic peak in A. The reactions contained 2 μl of each pool of fractions from peak I (fractions 39–47; lane 4), peak II (fractions 48–57; lane 5), peak III (fractions 58–70; lane 6), and peak IV (fractions 71–83; lane 7). In combination of pools, 2 μl of each were used as indicated at the top. D. poly(A) addition assay to determine the fractions with maximal CF IA and CF IB activities. To assay for CF IA, 2 μl of each fraction indicated at the top were mixed with 2 μl of the peak III pool (CF IB). To assay for CF IB, 2 μl of each fraction indicated at the top were mixed with 2 μl of the peak II pool (CF IA). E. SDS-polyacrylamide gel of the Superdex 200 fractions. Aliquots of 12 μl from every third fraction (lanes 3–13), as well as size markers (lane 1) and 2 μl of input material (lane 2), were separated on a 10% polyacrylamide gel and stained with silver. The molecular masses of size standards (in kilodaltons) are indicated on the left. The migration positions of four polypeptides that copurify with CF IA on a subsequent poly(A)-Sepharose column are indicated (C).
proteins are also present in CF IA from gel filtration fraction 48, the one with maximal activity (Fig. 6, lanes 4 and 11). The purified CF IA activity after the poly(A)-Sepharose step also contains both RNA14p and RNA15p (Fig. 6, lanes 5 and 10). The amount of RNA15p appears reduced in this fraction compared with that of RNA14p. ImmunobLOTS containing the gel filtration fractions show that both RNA14p and RNA15p peak in fractions 63 (Fig. 6, lane 1) and purified CF IB (10 μl of the fraction that eluted from the S-Sepharose column with 500 mM KCl) (lane 2) were separated on an 8% polyacrylamide gel and stained with silver. The arrows on the right of B and C indicate the position of the polypeptide that copurifies with CF IB.

RNA14p and RNA15p Are Tightly Associated—CF I-containing fractions from the phosphocellulose and heparin-Sepharose steps or CF IA after separation from CF IB by gel filtration was subjected to immunoprecipitation using antibodies against RNA15H6p covalently coupled to protein A beads. Analysis of the immunoprecipitated proteins on SDS-polyacrylamide gels stained with silver shows only the presence of RNA14p and RNA15p in the pellet (Fig. 7A, lanes 5–7) regardless of the source. This result suggests that a tight complex exists between RNA14p and RNA15p and that the other two proteins, which cofractionate with CF IA as well as CF IB, are washed away with the contaminants present in the fractions. The immunoprecipitation does not completely remove RNA14p and RNA15p from the input as these proteins are observed in the supernatants (Fig. 7A, lanes 2–4). The 70- and 50-kDa proteins, which cofractionate with CF IA, are also visible in the CF IA and heparin-Sepharose supernatants (Fig. 7A, lanes 3 and 4). The immunoprecipitation of RNA14p and RNA15p is specific because preimmune serum does not bring down either protein (Fig. 7B, lane 9). The identity of the precipitated RNA14p and RNA15p was confirmed by immunostaining a blot containing the pellet with a mixture of antibodies against RNA14H4p and RNA15H4p (Fig. 7C, lane 11).

The pellets from these immunoprecipitations did not exhibit cleavage activity when combined with CF II or poly(A) addition activity when combined with PF I and PAP (data not shown). The same results were obtained if CF IB was also included in the reaction. The lack of activity reflects the loss of the other protein components of CF I, destruction of CF I activity by the immunoprecipitation, or inhibition of activity by the anti-RNA15p antibodies. These coprecipitation results indicate that the RNA14p and RNA15p subunits of CF I form a very stable complex.

RNA15p Can Be Cross-linked to Substrate RNA—To test if RNA14p and RNA15p can bind the precursor RNA, radioactive RNA substrates were incubated with either protein fraction in the presence of tRNA as unspecific competitor at 30 °C. The mixture was then irradiated with UV light, treated with RNase A, and resolved on SDS-polyacrylamide gels. A 38-kDa protein present in CF IA was cross-linked to both wild type and mutant precleaved RNAs (Fig. 8A, lanes 1 and 2). No proteins were tagged with radioactive RNA if purified CF IB (S-Sepharose)
The combined mass of the CF IA subunits is ~240 kDa, in agreement with its elution position from the gel filtration column relative to the markers (~300 kDa). The 73-kDa CF IB protein eluted from the gel filtration column with an approximate molecular size of 100 kDa. The two components of CF I that separated in a glycerol gradient were of 9 and 4.5 S (corresponding to molecular masses of 190 and 65 kDa, respectively, assuming they are spherical globular proteins), again indicating that CF I fractionates into a large and a small component.

**DISCUSSION**

We have described the purification of yeast CF I and its separation into two parts, CF IA and CF IB. CF IA and CF IB are both required for cleavage and poly(A) addition, the two reactions in which CF I participates. CF IA copurifies with four polypeptides with molecular masses of 76, 70, 50, and 38 kDa, two of which are RNA14p and RNA15p. CF IB copurifies with a 73-kDa polypeptide.

The combined mass of the CF IA subunits is ~240 kDa, in agreement with its elution position from the gel filtration column relative to the markers (~300 kDa). The 73-kDa CF IB protein eluted from the gel filtration column with an approximate mass of 100 kDa. The two components of CF I that separated in a glycerol gradient were of 9 and 4.5 S (corresponding to molecular masses of 190 and 65 kDa, respectively, assuming they are spherical globular proteins), again indicating that CF I fractionates into a large and a small component.

CF I functions in both the cleavage and poly(A) addition reactions and is therefore the functional analog of mammalian CPSF. However, yeast CF I behaves differently than multisubunit CPSF in that the complex separates under conditions where CPSF stays intact. For example, CPSF does not break apart when chromatographed on a gel filtration column or when sedimented in a glycerol gradient (28, 29). Yeast CF I, however, separates during both of these treatments. CPSF also stays together when chromatographed on a blue Sepharose column (28) with similar properties to the Affi-Gel blue column, which separated CF I in this study. In addition, the FIP1 subunit of yeast PF I has some sequence similarity to the 160-kDa subunit of mammalian CPSF, suggesting that in yeast, a structural equivalent of the largest subunit of CPSF resides in PF I (9). This is interesting since yeast PF I participates only in poly(A) addition (11), while mammalian CPSF is required for both cleavage and poly(A) addition (29).

Even though yeast CF I appears to be functionally analogous to mammalian CPSF, its RNA14p and RNA15p subunits share sequence homology with the 77- and 64-kDa subunits of mammalian CstF (7). RNA14p and RNA15p are both components of CF IA, and they exist in a very stable complex as indicated by the cophoretic experiments. A similar situation occurs with mammalian CstF, which contains three polypeptides in a tight complex that cannot be reconstituted if the individual subunits are expressed separately and mixed (7). In addition, all three subunits of CstF sediment together in a glycerol gradient and can be immunoprecipitated together (30). However, CF I behaves differently in that only RNA14p and RNA15p are coimmunoprecipitated, and the other CF I subunits are washed away.

Genetic evidence provided the first indication of a complex between RNA14p and RNA15p since overexpression of wild type RNA14p or RNA15p could suppress *rnl15* and *rnl14* mutations, respectively (15). In addition, processing extracts...
made from yeast cells bearing a temperature-sensitive rna14 mutation did not complement extracts made from rna15 cells (16). This suggests that a functional RNA14p cannot replace a defective RNA14p in the complex in vitro. The same is true for RNA15p.

The separated CF IA and CF IB are both necessary for cleavage of full-length RNA as well as poly(A) addition, and they can only add poly(A) to GAL7 substrate RNA that contains a repeat of (UA)$_6$ residues upstream of the poly(A) site. This substrate specificity is observed with the ammonium sulfate extract (11,31) and with the CF I originally described by Chen and Moore (11). The UA repeat and similar motifs are important for the efficiency of the polyadenylation reaction, while a second element, located between the UA repeat and the cleavage site, positions the poly(A) site (32–34).

RNA15p is the only subunit of CF I that can be cross-linked to GAL7 substrate RNA, and this cross-linking does not require the presence of any other 3' end-processing factors. This is especially significant since RNA15p contains a potential RNA-binding domain in its N-terminal region (1,7,15). Interestingly, the mutant RNA lacking the (UA)$_6$ repeat was also cross-linked to RNA15p, even if the reaction mixture contained both CF IA and CF IB (data not shown). The RNA-binding motif found in RNA15p closely resembles the one found in the 64-kDa subunit of mammalian CstF (7), which recognizes the G/U-rich downstream element that is part of the mammalian core poly(A) signal (35). The efficiency with which the 64-kDa subunit cross-links to the polyadenylation precursor and the stability of the CstF-RNA complex are greatly enhanced by the binding of CPSF to the upstream AAUAAA sequence (9,29,36).

In a similar way, the substrate specificity of CF IA may be increased by components of CF II or PF I. This can be tested once these factors are purified and characterized. Alternatively, RNA15p may not interact with the (UA)$_6$ repeat of GAL7 RNA, but with another sequence, perhaps the positioning element that is presumed to be located 14 nucleotides upstream of the GAL7 poly(A) site (33) and is present in both wild type and mutant RNAs used in this study.

Once the yeast polyadenylation factors are purified and the genes encoding them cloned, the interaction of the various protein components with each other and the precursor RNA can be better understood. It will be interesting to identify the endonuclease that participates in cleavage and to compare the yeast processing factors with the mammalian machinery.

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