Six different protein factors are required for the specific cleavage and polyadenylation of pre-mRNA in mammals. Whereas four of them have been purified and most of their components cloned, cleavage factor IIm (CF IIm) and cleavage factor IIIm (CF IIIm) remained poorly characterized. We report here the separation of CF IIm from CF IIIm and the purification of CF IIm to near homogeneity. Three polypeptides of 68, 59, and 25 kDa copurify with CF IIm activity. All three polypeptides can be UV cross-linked to a cleavage and polyadenylation substrate in the presence of a large excess of unspecific competitor RNA, but not to a splicing-only substrate. No additional protein factor is required for the binding of CF IIm to pre-mRNA. Gel retardation experiments confirmed the results obtained by UV cross-linking. In addition, we could show that CF IIm stabilizes the binding of the cleavage and polyadenylation specificity factor (CPSF) to pre-mRNA and that CPSF and CF IIm together form a slower migrating complex with pre-mRNA than the single protein factors. Cleavage stimulation factor (CstF) and poly(A)polymerase (PAP) had no detectable effect on the binding of CF IIm to pre-mRNA. Furthermore, the CstF-CPSF-RNA as well as the CstF-CPSF-PAP-RNA complex are supershifted and stabilized upon the addition of CF IIm.

The 3' ends of almost all mRNAs in eukaryotes are generated posttranscriptionally in two tightly coupled steps. The primary transcript is first cleaved endonucleolytically at the polyadenylation site in the 3'-untranslated region. The upstream cleavage fragment is subsequently polyadenylated, whereas the downstream cleavage fragment is rapidly degraded. The two reaction steps can be uncoupled experimentally and asayed separately. Investigation of the cleavage reaction alone is possible by suppression of the Mg2+-dependent polyadenylation of the upstream cleavage fragment. No polyadenylation occurs, when the reaction is performed either in the presence of EDTA and ATP or in the presence of MgCl2 and cordycepin 5'-triphosphate (3'-dATP) which acts as a chain terminator. The polyadenylation reaction can be investigated with “pre-deaved” RNA as substrate that ends at or near the natural cleavage and polyadenylation site.

Both cleavage and polyadenylation are dependent on cis-acting elements in the pre-mRNA and on trans-acting protein factors (for reviews, see Refs. 1-4). Two essential cis-acting elements have been described: one of them, the highly conserved polyadenylation signal AAUAAA, lies 10–30 nucleotides upstream of the cleavage and polyadenylation site, and the second one, a less conserved GU- or U-rich sequence element, is located 10–30 nucleotides downstream of the cleavage and polyadenylation site.

Six different protein factors have been shown to be required for specific cleavage and polyadenylation of pre-mRNA in vitro (5, 6). Three of them, cleavage stimulation factor (CstF), cleavage factor IIm (CF IIm) and cleavage factor IIIm (CF IIIm; abbreviations for CF IIm and CF IIIm, according to (3)) are involved in the cleavage reaction only, while the cleavage and polyadenylation specificity factor (CPSF) and poly(A)polymerase (PAP) are necessary for both steps. Poly(A)-binding protein II (PAB II) acts as a stimulatory factor for poly(A) tail elongation. CstF, CPSF, PAP, and PAB II have been studied extensively, are well characterized, and most of their components have been cloned. CPSF consists of three subunits. Its 64-kDa subunit was shown to interact with the GU- or U-rich downstream sequence element (7–11). CPSF consists of three or four subunits (12–15) and binds specifically to the polyadenylation signal AAUAAA (12, 16). CstF and CPSF, together with a pre-mRNA substrate, form a stable complex (17) and are thought to confer specificity to the 3′ end processing reaction. The approximate region in which cleavage will occur is defined by the relative positions of the AAUAAA and the GU- or U-rich elements, and the precise site of cleavage is then determined by a preference for a local nucleotide sequence within this region (18). CPSF remains bound to the hexanucleotide AAUAAA after cleavage has occurred and enables PAP to elongate specifically the upstream cleavage fragment to a poly(a) tail length of 250 nucleotides in the presence of PAB II (19).

The endonuclease could not be identified so far. Good candidates are the two poorly characterized cleavage factors CF IIm and CF IIIm, which are required only for the first step of the reaction.

We report here the separation of CF IIm from CF IIIm and the purification of CF IIm to near homogeneity. Each of the three polypeptides copurifying with CF IIm activity can be UV cross-linked to a cleavage and polyadenylation pre-mRNA substrate and gel retardation experiments showed that CF IIm binds to pre-mRNA, even in the absence of any of the other protein factors involved in 3′ end processing of pre-mRNA.

**EXPERIMENTAL PROCEDURES**

RNAs—RNAs used as substrates for cleavage reactions, UV cross-linking, and gel retardation assays were prepared from the following plasmids: plasmid pSV-L contains the polyadenylation signal of the SV40 late transcription unit (20), and plasmids pSP6L.3 and pSP6L.3Δ1 contain the L3 polyadenylation signal of the adenovirus 2 major late
transcription unit carrying either the wild-type polyadenylation signal AAGAAA or the mutated polyadenylation signal AAGAGA, respectively (21). Plasmid pSP63pre was derived from pSP63 by truncation of the L3 polyadenylation signal one nucleotide upstream of its natural cleavage site (22). Plasmid pBAD1 contains the first two leader exons, the shortened form of the first intron, and 27 nucleotides of the second intron of the adenovirus 2 major late transcription unit (23).

Plasmids pSV-L, pSP63, and pSP63.311 were linearized with DraI, pSP63pre with RsaI, and pBAD1 with Sau3A I. Capped, uniformly 32P-labeled RNAs were obtained by in vitro transcription of the linearized template DNAs with SP6 RNA polymerase (Boehringer Mannheim GmbH), in the presence of 0.1 M of GDP's, GTP'S, ATP, and x-32P JUTP as described (24, 25), except that the UTP concentration was 0.1 M.

16 and 23 S rRNA and tRNA from Escherichia coli MRE 600 were purchased from Boehringer Mannheim GmbH.

Proteins—CsF was purified from HeLa cell nuclear extracts as described (26). CPSF was prepared from calf thymus according to a procedure described (26). CPSF was prepared from calf thymus according to a procedure described (26).

Cleavage Assays—Cleavage reactions (25 ml) were assembled on ice as follows: 10 ml of premix (5 mM dithiothreitol, 0.025% (v/v) Nonidet P-40, 50 mM creatine phosphate (Boehringer Mannheim GmbH), 6.5% (w/v) polyvinyl alcohol, 0.5 unit/mL RNAguard (Pharmacia), 0.025% (v/v) creatine kinase (Boehringer Mannheim GmbH), 1.25 mM cordycepin 5'-triphosphate (Boehringer Mannheim GmbH), 3.75 mM MgCl2) were mixed with 60 fmol of CsFsF, 50 fmol of CPSF, 240 fmol of PAP, 4 ml of crude CF Im, and the amount of CF Im indicated. The volume was adjusted to 23.5 ml with buffer E containing 50 mM Tris-HCl, pH 7.9, 10% (v/v) glycerol and 0.1 mM Na2-EDTA. The final ammonium sulfate concentration was kept below 20 mM. The reaction was started on ice by the addition of 7.5 fmol of 32P-labeled L3WT pre-mRNA reservoir and product. Cleavage activity was calculated by dividing the amount of 32P-labeled RNA in the downstream cleavage product by the sum of upstream cleavage product and precursor and multiplying the ratio by the amount of precursor added to the reaction. 1 unit corresponds to 1 fmol of upstream cleavage product obtained during the incubation time and at the temperature indicated.

UV Cross-linking of 32P-Labeled 30S pre-mRNA—Reactions (12.5 ml) were assembled on ice as follows: 5 ml of premix (5 mM dithiothreitol, 0.025% (v/v) Nonidet P-40, 2.5% (w/v) polyvinyl alcohol, 0.5 unit/mL RNAguard (Pharmacia), 1.25 mM cordycepin 5'-triphosphate (Boehringer Mannheim GmbH), and 2.5 mM Na2-EDTA) were mixed with the protein fractions indicated and the volume adjusted to 10.5 ml with buffer E as described for cleavage assays. After the addition of 7.5 fmol of 32P-labeled 30S pre-mRNA reservoir and product, the reaction was assembled on ice. The reaction was incubated for 30–35 min at 30 °C. 10 ml of the reactions were loaded in separate lanes, and electrophoresis was carried out until the xylene cyanol dye marker had reached the bottom (27, 28). To quantitate cleavage reactions, gels were exposed to Phosphor Imager screens for 1 h, the screens were scanned with a Phosphor Imager (Molecular Dynamics) and the ImageQuant program (version 3.3, Molecular Dynamics); the amount of precursor and upstream cleavage product was determined with the IPLab Gel software (version 1.5, Signal Analytics Corp.). The values obtained were corrected by subtraction of the background signal of the gel in a region where no radioactivity was detectable. The value for the upstream cleavage fragment was further corrected for the different uridine contents of precursor and product. Cleavage activity was calculated by dividing the amount of upstream cleavage product by the sum of upstream cleavage product and precursor and multiplying the ratio by the amount of precursor added to the reaction. 1 unit corresponds to 1 fmol of upstream cleavage product obtained during the incubation time and at the temperature indicated.

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mixed with the \(^{32}\)P-labeled substrate prior to addition to the reaction and to purify CF Im are shown schematically. The gradients applied to cleavage factor Im.

To analyze the cleavage factors further, we separated CF Im from more active when the nuclei were extracted with 200 mM ammonium sulfate instead of 300 mM KCl. Since this effect might be due to a stabilization of proteins by ammonium sulfate, all columns were eluted with ammonium sulfate, even though already small concentrations of ammonium sulfate (\(\approx 30\) mM) inhibit the cleavage reaction significantly (data not shown).

When diluted nuclear extracts were applied to a DEAE-Sepharose column, CstF and PAP did not bind to the column, whereas CF Im and CF II m and CPSF bound and were eluted with a salt gradient. CF Im and CF II m were probably partially separated on this column, since a pool of the fractions showing cleavage activity was more active than the single fractions (data not shown). CF Im and CF II m were separated in the next purification step, a Mono S column, as described previously (5). The profile of the Mono S column is shown in Fig. 2. All column fractions were assayed for CF Im and CF II m activity in the presence of CstF, CPSF, PAP, and crude CF II m or crude CF Im respectively. Crude CF Im and CF II m used for complementation in these assays were obtained from a 1-ml pilot Mono S column and had been identified before by testing all possible fraction combinations. The elution positions of CF Im and CF II m from the Mono S column described here are indicated by horizontal bars above the column profile (Fig. 2).

By means of ammonium sulfate precipitation and two additional chromatographic steps (see Fig. 1 and “Experimental Procedures”), CF Im was purified to near homogeneity. The purification is summarized in Table I. The quantitation of cleavage reactions proved to be difficult for the first steps of the purification. For obvious reasons, the calculation of activities does not give reliable numbers only after the separation of CF Im and CF II m. The analysis of the separation of CF Im and CF II m on Mono S was complicated for two reasons: first, a nuclease interfered with the determination of CF Im activity and second, maximal cleavage activity depended on the optimal ratio of CF Im and CF II m at this stage of purification. Only after further purification of CF Im did cleavage activity depend linearly on the amounts of CF Im and CF II m (data not shown). Therefore, the elution positions of CF Im and CF II m are indicated only qualitatively in Fig. 2.

The profile of the final Mono Q column is shown in Fig. 3A. Three polypeptides with apparent molecular masses of 68, 59, and 25 kDa copurified with CF Im activity (Fig. 3, B and C). The fractions were also tested for RNA binding by gel retardation (Fig. 3D, see below). No RNA component could be detected in CF Im fractions of the Mono Q column after proteinase K treatment and 3' end labeling with [\(\alpha\)\(^{32}\)P]cordycepin 5'-triphosphate and PAP (30) (data not shown). On this column, the 68-kDa polypeptide eluted slightly later than the 59- and 25-kDa polypeptides, and the staining was less intense. Independent purifications over the same or different columns

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**RESULTS**

**Purification of Cleavage Factor Im**—Six different protein factors have been shown to be required for specific cleavage and polyadenylation of mammalian primary mRNA transcripts. Whereas four of them, CstF, CPSF, PAP and PAB II, have been purified and most of their components cloned, the two others, CF Im and CF II m, remained poorly characterized. In order to analyze the cleavage factors further, we separated CF Im from CF II m on Mono S as described (5) and purified CF Im to near homogeneity. Column fractions were assayed for reconstitution of endonucleolytic cleavage of pre-mRNA derived from the polyadenylation site of the SV40 late transcription unit (SV40 pre-mRNA) in a reconstitution system containing CstF purified from HeLa cell nuclear extracts, CPSF isolated from calf thymus, recombinant bovine PAP and, after the separation of CF Im and CF II m, crude CF II m. The assays were performed in the presence of cordycepin 5'-triphosphate and MgCl\(_2\), since under these conditions endonucleolytic cleavage of the SV40 pre-mRNA was much more efficient than in the presence of ATP and EDTA. Although PAP was not essential for cleavage of the SV40 pre-mRNA to occur, it stimulated the reaction at least 2-fold (data not shown).

CF Im was purified from HeLa cell nuclear extracts by several fractionation steps, as shown schematically in Fig. 1. Nuclear extracts from HeLa cells turned out to be at least 50% more active when the nuclei were extracted with 200 mM ammonium sulfate instead of 300 mM KCl. Since this effect might be due to a stabilization of proteins by ammonium sulfate, all columns were eluted with ammonium sulfate, even though already small concentrations of ammonium sulfate (\(\approx 30\) mM) inhibit the cleavage reaction significantly (data not shown).

When diluted nuclear extracts were applied to a DEAE-Sepharose column, CstF and PAP did not bind to the column, whereas CF Im and CF II m and CPSF bound and were eluted with a salt gradient. CF Im and CF II m were probably partially separated on this column, since a pool of the fractions showing cleavage activity was more active than the single fractions (data not shown). CF Im and CF II m were separated in the next purification step, a Mono S column, as described previously (5). The profile of the Mono S column is shown in Fig. 2. All column fractions were assayed for CF Im and CF II m activity in the presence of CstF, CPSF, PAP, and crude CF II m or crude CF Im respectively. Crude CF Im and CF II m used for complementation in these assays were obtained from a 1-ml pilot Mono S column and had been identified before by testing all possible fraction combinations. The elution positions of CF Im and CF II m from the Mono S column described here are indicated by horizontal bars above the column profile (Fig. 2).

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The profile of the final Mono Q column is shown in Fig. 3A. Three polypeptides with apparent molecular masses of 68, 59, and 25 kDa copurified with CF Im activity (Fig. 3, B and C). The fractions were also tested for RNA binding by gel retardation (Fig. 3D, see below). No RNA component could be detected in CF Im fractions of the Mono Q column after proteinase K treatment and 3' end labeling with [\(\alpha\)\(^{32}\)P]cordycepin 5'-triphosphate and PAP (30) (data not shown). On this column, the 68-kDa polypeptide eluted slightly later than the 59- and 25-kDa polypeptides, and the staining was less intense. Independent purifications over the same or different columns
showed either the same effect or resulted in exactly comigrating polypeptides with an approximate equimolar ratio. Two-dimensional gel electrophoresis on a nondenaturing polyacrylamide gel (31) in the first dimension and a denaturing SDS-polyacrylamide gel in the second dimension showed that the three polypeptides comigrated on the native gel (results not shown). These and other data suggest that all three polypeptides are part of CF Im (see below).

**Table 1**

| Step                  | Protein | Activity | Specific activity | Yield | Purification |
|-----------------------|---------|----------|-------------------|-------|--------------|
|                       | mg      | units    | units/mg          | %     | -fold        |
| Nuclear extract       | 900     |          |                   |       |              |
| DEAE-Sepharose        | 147     |          |                   |       |              |
| Mono S                | 45      | 59,700   | 1300              | 100   |              |
| AS fractionation      | 11      | 62,600   | 5700              | 105   | 4.4          |
| Phenyl-Superose       | 0.5     | 12,100   | 24,200            | 20    | 18.6         |
| Mono Q                | 0.175   | 10,100   | 57,700            | 17    | 44.4         |

**FIG. 3. Chromatography of CF Im on Mono Q.**

A. profile of the final Mono Q column of the preparation summarized in Table I. B, SDS-polyacrylamide gel electrophoresis of Mono Q fractions. Aliquots of 2 μl of the fractions indicated at the bottom were separated on two 10% gels and stained with silver. The molecular masses of the size standards in kilodaltons are indicated on the left. Arrowheads on the right indicate the three polypeptides copurifying with CF Im activity. L, load of the Mono Q column. C. Cleavage of SV40 late pre-mRNA. Assays were carried out as described under "Experimental Procedures" for 85 min at 30 °C with 1 μl of the fractions indicated at the bottom. Samples were analyzed on two denaturing 6% (w/v) polyacrylamide gels. Sizes (in nucleotides) of DNA size standards (lane M) are indicated on the left. The migration behavior of the SV40 late pre-mRNA substrate and the upstream cleavage product are indicated on the right. R, SV40 pre-mRNA incubated without protein fractions; –, SV40 pre-mRNA incubated in the presence of CstF, CPSF, PAP, and crude CF II m; L, load of the Mono Q column. D, gel retardation assay of Mono Q fractions. Aliquots of 2 μl of the fractions indicated at the bottom were preincubated with 7.5 fmol of uniformly 32P-labeled L3 pre-mRNA in the presence of tRNA as unspecific competitor, and the reactions were loaded directly on a native polyacrylamide/agarose composite gel. The migration positions of the free pre-mRNA, and the protein-RNA complexes are indicated at the left. R, RNA incubated without protein fractions; L, load of the Mono Q column. For details, see "Experimental Procedures."
the presence of heat denatured rRNA, which was added to the reaction simultaneously with L3 pre-mRNA and tRNA as a less structured unspecific competitor (compare lanes 3 and 7). Weak cross-links of the 64-kDa subunit of CstF could also be detected to the L3a1 substrate that carries a point mutation in the AAUAAA polyadenylation signal and is thus no longer able to bind CPSF (lane 11), and to the L3pre RNA that ends one nucleotide upstream of the natural cleavage and polyadenylation site of the L3 pre-mRNA and lacks the natural binding site for CstF (L3pre; lane 15). The unspecific cross-linking of CstF to RNA can probably be explained by the observation that CstF alone has no strict sequence requirements for binding to RNA and that CstF-RNA complexes are stabilized by CPSF and PAP, respectively. No cross-link could be detected to the splicing substrate Ad1, which does not contain a polyadenylation signal (lane 19).

The same set of RNA substrates was used for UV cross-linking reactions with CF Im. The peak fraction of the Mono Q column shown in Fig. 3 (fraction 37) was irradiated with UV light either alone or in the presence of CstF, CPSF, and PAP. Surprisingly, all three polypeptides of CF Im were cross-linked to the L3 pre-mRNA in the absence of any other 3’ end processing factor (lane 4). The cross-links were assigned to the three polypeptides by superimposing the autoradiograph and the silver-stained gel; the migration positions of the 68-, 59-, and 25-kDa polypeptides of CF Im on the silver-stained gel are indicated by arrowheads in Fig. 4. The 68- and 59-kDa polypeptides comigrated exactly with signals detected on the autoradiograph, whereas the 25-kDa polypeptide detected by silver staining migrated slightly faster than the signal detected by autoradiography. This is probably due to retardation of the cross-linked portion of the polypeptide by covalently bound residual RNA nucleotides. The difference in the migration behavior is detectable for small polypeptides like the 25-kDa polypeptide but not for larger ones such as the 59- and 68-kDa polypeptides of CF Im.

The signal of the cross-link of the 68-kDa polypeptide is weaker than the signal of the 59-kDa polypeptide. The cross-linking efficiency of proteins to RNA largely depends on the amino acid composition of the RNA binding site and, since the RNA substrate was labeled with [α-32P]UTP, also on the uridine content of the protein binding site on the RNA. The cross-links of CF Im to L3 pre-mRNA were neither reduced by the addition of rRNA as unspecific competitor (lane 8) nor were they affected by a point mutation in the AAUAAA polyadenylation signal (lane 12); however, they were not detectable with the precleaved substrate L3pre (lane 16) nor with the splicing substrate Ad1 (lane 20). The cross-linking efficiency was not enhanced by the addition of CstF, CPSF, and PAP (compare lanes 4 with 5, 8 with 9, and 12 with 13).

CF Im Stabilizes the CPSF-RNA Complex—For gel retardation assays, aliquots from the Mono Q column shown in Fig. 3 were preincubated with L3 pre-mRNA for 30 min at 30 °C and loaded directly onto a native polyacrylamide/agarose composite gel at 4 °C. Reactions were done under similar conditions as cleavage assays, but contained less polyvinyl alcohol, and EDTA instead of MgCl2 (see “Experimental Procedures”). The autoradiograph of the dried gel is shown in Fig. 3D. The protein-RNA complex comigrated exactly with CF Im activity, but only CF Im peak fractions gave rise to a complex detectable as a distinct band. Smaller amounts of CF Im resulted in apparently less stable protein-RNA complexes. The faint band detectable in almost all lanes migrating above the protein-RNA complexes most likely results from a RNA with a different structure.

In order to compare the ability of all known 3’ end processing factors to bind RNA in gel retardation experiments, equal amounts (650 fmol) of CF Im, CstF, CPSF, and PAP were incubated separately with 7.5 fmol of 32P-labeled L3 pre-mRNA and run on a native gel (Fig. 5). Even with this high excess of protein, only CPSF formed a distinct complex with the pre-mRNA substrate (lane 4), whereas CF Im RNA and CstF-RNA complexes formed short smear running above the free RNA (lanes 2 and 3). PAP also did not shift the L3 pre-mRNA at all (lane 5). Normally, much smaller concentrations of CstF (30 fmol in 12.5 μl), CPSF (25 fmol in 12.5 μl), and PAP (120 fmol in 12.5 μl)
polypeptides formed a complex that was stable upon native gel electrophoresis. 3) The three polypeptides copurified during different purification procedures, although they were partially separated on certain column matrices. We, therefore, believe that all three polypeptides are part of CF Im, but we cannot rule out at this stage that the 59-kDa and/or 25-kDa polypeptide are degradation products of the 68-kDa polypeptide that are still able to bind to RNA and are at least partially active. Partial proteolysis of the 68-kDa polypeptide would be one possible explanation for the observation that the largest subunit was slightly less abundant than the other two polypeptides in some CF Im preparations. Attempts to reconstitute CF Im from the single polypeptides obtained by elution from a SDS-polyacrylamide gel were unsuccessful. Thus, the true composition of CF Im remains uncertain until cDNAs coding for the polypeptides will be cloned.

The question whether CF Im can bind to RNA either alone or in the presence of other 3′ end processing factors was addressed by two different methods, UV cross-linking and gel retardation assays. Whereas by UV cross-linking even weak interactions of proteins and RNA can be detected, and the RNA binding polypeptide(s) can be identified on a SDS-polyacrylamide gel, gel retardation assays can reveal protein-RNA interactions in solution. Both methods allowed the detection of a CF Im-RNA complex in the absence of any other 3′ end processing factor. UV cross-links of CF Im could only be detected to the cleavage and polyadenylation substrate L3, but not to the splicing substrate Ad1. The preference of CF Im for L3 was in principle confirmed by gel retardation assays, but the CF Im-L3 shift could be competed by increasing amounts of Ad1, although less efficiently than by L3 itself (data not shown). No cross-links of CF Im to L3pre mRNA, which ends one nucleotide upstream of the natural cleavage and polyadenylation site, could be detected. It is not possible to decide from the experiments shown here where precisely CF Im binds on L3 pre-mRNA. Further experiments are needed to map the region on the pre-mRNA substrate that is bound by CF Im.

Gel retardation assays with purified CstF, CPSF, PAP, and crude cleavage factors, either alone or in different combinations, and L3 pre-mRNA have been described before (13, 16, 17, 19). It was shown that formation of the CPSF-RNA complex depends on AAUAAA, that the CPSF-RNA complex is stabilized by PAP, and that addition of CstF causes the formation of a slower migrating complex. Under the conditions used, neither a CstF-RNA nor a PAP-RNA complex could be detected. These results are in good agreement with those presented here, which were obtained with more highly purified components. We further demonstrated the binding of CF Im to L3 pre-mRNA in the absence of any other protein factor. Neither CstF nor PAP had an influence on the binding of CF Im to L3 pre-mRNA, but the addition of CPSF led to the formation of a more slowly migrating complex. Furthermore, CF Im stabilized the Cstf-CPSF-RNA complex as well as the Cstf-CPSF-PAP-RNA complex and resulted in slower migrating complexes. In contrast, Gilmartin and Nevins (17) have reported previously that the addition of crude cleavage factors abolished the formation of the CPSF-RNA complex and destabilized the Cstf-CPSF-RNA complex. The results presented here were obtained in the absence of CF Im, because this factor is presently only available as a relatively impure fraction. The destabilization of the CPSF-RNA and the Cstf-CPSF-RNA complex by the cleavage factors (17) may thus be either an unspecific effect, since less purified cleavage factors were used in these experiments, or it may have been caused by CF Im.

The results obtained from the UV cross-linking experiments and gel retardation assays are consistent with the suggestion...
that CF \textsubscript{Im} binds to pre-mRNA with a certain degree of specificity and interacts with CPSF, but not with CstF and PAP upon binding to RNA.

It remains to be shown which of the protein factors essential for the cleavage reaction acts as the actual endonuclease. This question can only be resolved after the other cleavage factor, CF II\textsubscript{m}, has been characterized. The purification of all factors involved in cleavage and polyadenylation of primary mRNA transcripts should allow it to study in more detail the assembly of the components involved in 3’ RNA processing, to investigate the sequence requirements further, and to identify regulatory factors that participate in the 3’ end formation of pre-mRNA in a well defined, fully reconstituted system.

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