The yeast Mediator complex is required for transcription by RNA polymerase II (pol II) in vivo and in vitro. This complex of over 20 polypeptides associates with pol II and is recruited to transcription complexes at promoters. Previous isolations of yeast Mediator-containing complexes in different laboratories have identified several distinct complexes. To identify the major forms of Mediator in yeast, Mediator was isolated from nuclear extracts using a two-step chromatographic procedure, avoiding ion exchange chromatography and high salt conditions to prevent dissociation of subunits during purification. Components of the Mediator complexes were identified by mass spectrometry and Western analysis. The major form of Mediator, termed pol II Med, contained pol II and Mediator, including the Srb8–11 module. A second lower molecular size complex was also identified, termed Mediator core (Medc), which lacked pol II, Srb8–11, Rox3, Nut1, and the Rgr1 module. Both of these complexes were active in transcription in vitro, although the Medc complex had significantly lower activity and could compete with the activity of the pol II-Med complex in vitro.

Transcription of protein-coding genes requires RNA polymerase II (pol II) as well as six general transcription factors termed TFIIA, TFIIB, TFIIF, TFIIH, and TFIIF. Together these factors comprise the minimal pol II transcription machinery and can promote accurate transcription initiation from a core promoter (1, 2). However, additional factors termed coactivators are required for response to transcriptional regulatory signals. These factors include the chromatin remodeling machinery, histone acetylases, the TBP-associated factors, and the Mediator complex (1, 3–7). The Mediator complex was first discovered in yeast and consists of more than 20 polypeptides. The Srb2, -4, -6, and -6 Mediator subunit genes were identified as dominant suppressors of deletions in the C-terminal domain (CTD) of the largest pol II subunit (8). Likewise, the Srb7–11 subunits were identified as recessive suppressors of CTD deletions. The remaining subunits were identified by biochemical fractionation and include proteins previously identified as having positive and/or negative roles in gene regulation (Hrs1/Pgd1, Gal11, Sin4, Nut1, Nut2, Rox3, and Rgr1) and other subunits not previously known to be involved in transcription (Med1, -2, -4, -6, -7, -8, -9, and -11) (9–11).

In vivo, and in crude nuclear transcription extracts, the yeast Mediator behaves functionally as a general transcription factor. Heat shock of a strain containing a temperature-sensitive Srb4 allele results in a global decrease in pol II transcription equivalent to a mutation in pol II (12, 13). Likewise, transcription in vitro is highly dependent on Srb2, -4, and -6 in nuclear extracts (14, 15). In this nuclear extract system, mutation of Srb2, -4, or -6 blocks recruitment of factors except TFIIA and TFIIID to the preinitiation complex (PIC) (15). After transcription initiation, Mediator along with TFIIA, TFIIID, TFIIH, and TFIIIE remain at the promoter forming a scaffold for recruitment of pol II and other missing factors required for transcription reinitiation (16). Specific activators can stabilize this scaffold complex (16). In highly purified in vitro systems, Mediator stimulates both basal and activated transcription as well as phosphorylation of the pol II CTD (17–20). In these highly purified systems, Mediator isolated from strains carrying deletions of nonessential subunits is defective in response to specific transcriptional activators.

In the past several years, human homologs of the yeast Mediator complex have been identified by affinity purification, interaction with specific activators, and on the basis of coactivator activity. At least eight different human and mouse Mediator complexes have been identified (1, 3, 21). These complexes differ in their exact polypeptide composition but contain a common set of polypeptides, including homologs to yeast Rgr1 and Med7. Interestingly, even though Srb2, -4, -5, and -6 are crucial to yeast Mediator function, no homologs to these factors have yet been identified in any organism other than yeasts. Because both yeast and human Mediator subunits interact with specific transcription activators, a current model for activator function is that interaction between specific activators and specific Mediator subunits aids in recruitment of the transcription machinery to promoters (1, 3). Furthermore, transcription reinitiation may be facilitated by interactions between activators and Mediator subunits that may stabilize the scaffold complex (16).

Several groups have reported biochemical fractionation of yeast Mediator-containing complexes from whole cell extracts. Young and coworkers followed purification of Mediator subunits by Western blot assay and isolated a complex termed RNA pol II holoenzyme. This complex contains pol II, Mediator (including the Srb8–11 subunits), TFIIIB, TFIIIF, TFIIH, and
Mediator components, and based on other work, appears to be the Srb8–11 module. The other complex consists of a subset of Kornberg, Kim, and colleagues, but in addition contains the identified and found to be distinct from those complexes previously identified. The difference of TFIIIB signals in elutions from the three extracts is not significant owing to the poor linearity of the TFIIIB antibody signal.

In light of the different Mediator-containing complexes isolated by biochemical fractionation and the distinct modules of Mediator subunits identified by biochemical and genetic studies, we have investigated the different forms of Mediator complexes in yeast. To avoid high salt conditions and multiple ion-exchange chromatographic steps used previously that could cause similar methods involving ion exchange chromatography were used by all three groups (24, 25). Finally, using a different purification method, Jaehning and coworkers (26) have isolated a complex termed Pafl or alternative holoenzyme, which contains pol II, TFIIIB, TFIIIC, Cdc73, Pafl, Ccr4, Hpr1, and the Mediator subunits Gal11 and Sin4.

Both biochemical and genetic studies have suggested that the Mediator is composed of stable subcomplexes or modules. One module contains the dominant CTD suppressors Srb2, -4, -5, and -6 as well as Med6 and Rox3 (27, 28). This subcomplex is stable in 1 M urea. A second module consists of Rgr1, Gal11, Sin4, Hrs1/Pgd1, and Med2. Evidence for this subcomplex is that pol II-Med with a C-terminal deletion of Rgr1 lacks Gal11, Sin4, and Hrs1 (20, 29). Also, deletion of Gal11 causes loss of the Hrs1 subunit and vice versa (20). Deletion of the Sin4 subunit causes loss of both Med2 and Hrs1 and deletion of Med2 causes loss of the Hrs1 subunit (19). Another distinct module consists of Srb8–11, which acts genetically as a repressor of pol II (30). Deletion of any one of these Srb genes suppresses deletions in the pol II CTD, and deletion of the Srb8 subunit gene causes loss of Srb10 and -11 in the holoenzyme (30). Whole genome array analysis showed that deletion of Srb10 derepressed transcription from a subset of yeast genes (13).

In light of the different Mediator-containing complexes isolated by biochemical fractionation and the distinct modules of Mediator subunits identified by biochemical and genetic studies, we have investigated the different forms of Mediator complexes in yeast. To avoid high salt conditions and multiple ion-exchange chromatographic steps used previously that could cause similar methods involving ion exchange chromatography and gel filtration were used to isolate yeast Mediator complexes under mild conditions. Using these methods, two major yeast Mediator-containing complexes were identified and found to be distinct from those complexes previously identified. One complex is similar to the holoenzyme isolated by Kornberg, Kim, and colleagues, but in addition contains the Srb8–11 module. The other complex consists of a subset of Mediator components, and based on other work, appears to be the core Mediator complex.

**MATERIALS AND METHODS**

**Construction of FLAG-tagged Yeast Strains**—A PCR-mediated epitope-tagging method (31) was used to C-terminally tag the yeast Srb5, Srb4, and Rgr1 open reading frames. In brief, plasmid pBAD-FLAG-KanMX (32), which contains a tagging cassette (three copies of the FLAG epitope upstream of the KanMX marker) was used as the PCR template. Each pair of PCR primers contained 45 bases homologous to the target gene and 18 bases complementary to the tagging cassette. PCR products were transformed to yeast, and the tagging cassette was introduced into the target gene by homologous recombination. Correct colonies were selected on G418 plates and identified by colony PCR. FLAG-tagged proteins were confirmed by Western blotting.

**Extract Preparation and Purification of Protein Complexes**—The C-terminal FLAG-tagged strains were grown in YPD media, and nuclear extracts were prepared as described previously (33). 100 mg of nuclear extract (20–40 μg/ml of protein) was diluted to 30 ml with buffer A (20 mM HEPES-KOH, 10% glycerol, 0.01% Nonidet P-40, 2 mM DTT, and protease inhibitors) containing 0.25 mM KOAc and incubated with 1 ml of anti-FLAG M2-agarose beads (Sigma) at 4 °C for 4 h. After ten 30-ml washes with buffer A containing 0.3 mM KOAc, proteins were eluted twice from the beads by incubation at 4 °C for 1 h with buffer A containing 0.3 mM KOAc and 0.5 mg/ml FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) (synthesized by Research Genetics). The eluted proteins were concentrated by Centricron YM-30 (Millipore) and applied to a TSK-G4000SWxl (TosoHaas) gel filtration column at 0.37 ml/min equilibrated in buffer B (20 mM HEPES-KOH, pH 7.5, 0.25 mM EDTA, 0.3 mM KOAc, 0% glycerol, 0.01% Nonidet P-40, 1 mM DTT, and protease inhibitors). 135-μl fractions were collected, and 5 μl was used in Western blot analysis. Fractions containing protein complex were combined and concentrated by Microcon YM-10 (Millipore) for use in the transcription assay and immobilized template assay. For the estimation of molecular size of protein complexes, blue dextran (2000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), and bovine albumin (66 kDa) (Sigma) were loaded onto the same column as molecular size markers.

**Western Blotting**—All Western blots were performed as described (15). Proteins were detected with the following antibodies: anti-Srb2, Rpb3, TFIIIB, TBP (15), anti-Srb4, Srb10, TFG2 (J. Movius and K. Coachman, Fred Hutchinson Cancer Research Center), anti-Sin4 (D. Stillman, University of Utah), anti-Gal11 (H. Sakurai, Kanazawa University, Japan), anti-Kin28, Rpb1 (Berkeley Antibody Co.), and anti-FLAG M2 (Sigma). Quantitative Western blots were performed as described (15). Serial dilutions of recombinant proteins overexpressed and purified from *Escherichia coli* (*rSrb2, rSrb4, rSrb10, rRpb3*, TFIIIB, and TFG2) were used as standards. Band intensities were determined by densitometry using IQMAC v1.2 software (Molecular Dynamics).

**Mass Spectrometry Analysis of the pol II-Med and Medc Complexes**—Peak fractions corresponding to pol II-Med or Medc from the gel filtration column were pooled and concentrated using Microcon YM-10 (Milli-
TABLE I
Recovery of Mediator, pol II, and GTFs during affinity purification

| Proteins | Recovery % | Molecules/cell |
|----------|-------------|----------------|
| Mediator | 70          | 2–4,000        |
| Srb5, Srb6, Rgr1-FLAG | 70 | 2–4,000 |
| Srb4      | 70          |                |
| Gal11     | 70          |                |
| Pol II    |             |                |
| Rpb1      | 5–10        | 10–20,000      |
| GTF       |             |                |
| TFIIIB    | <1          | 30–50,000      |
| TFIIF     | <1          | 30–50,000      |
| TFIIH     | ~0.5        | N.D.~          |
| TBP       | ~0.5        | 30–50,000      |

~ Not detectable.
~ N.D., not determined.

RESULTS

Purification and Identification of Mediator Complexes—Previous biochemical fractionation of Mediator-containing complexes from yeast whole cell extracts have resulted in isolation of several complexes: the holoenzyme containing pol II, Srb8–11, and the general transcription factors TFIIH, TFIIF, TFIIF (18), the holoenzyme lacking Srb8–11 and all general transcription factors (11), the holoenzyme including a single general transcription factor TFIIF (17), and free Mediator lacking both Srb8–11 and pol II (36). These fractionation methods have all used multiple ion exchange chromatography steps and high ionic strength buffers. It is possible that some of the differences in Mediator-containing complexes isolated by different laboratories arise from dissociation of subunits under these conditions. For example, it has been shown that a fraction of Mediator can be separated from pol II by ion exchange gel (Invitrogen), and the composition of PICs was analyzed by quantitative Western blotting.

FIG. 2. Gel filtration chromatography of FLAG-tagged Srb5-associated proteins. The affinity-purified protein derived from the Srb5-tagged nuclear extract was subjected to gel filtration chromatography on a TSK-G4000SWXL column. Fractions were separated by SDS-PAGE in a 4–12% NuPAGE gel and analyzed by Western blotting with antibodies directed against the proteins indicated. The migration positions of molecular size markers were indicated as arrows. Serial dilutions of recombinant proteins were loaded as protein standards (S1–S4) in the following amounts: 120, 40, 13.3, and 4.4 fmol of rSrb4 and rRpb3; 200, 66.7, 22.2, and 7.4 fmol of rSrb2; 15, 5, 1.7, and 0.6 fmol of rTFIIH.

2 Available from the S. Hahn laboratory on the web.
chromatography (36). To isolate Mediator complexes under gentler conditions, we first epitope-tagged the genomic copy of the subunits Srb5, Srb6, or Rgr1 with three copies of the FLAG epitope at the C terminus of these genes. As judged by cellular growth rates compared with wild-type strains, and by in vitro transcription using extracts made from these strains, the FLAG epitopes did not affect the function of any of these Mediator subunits. As a source for Mediator purification, we used yeast nuclear extracts, because these extracts have a much higher specific transcription activity compared with whole cell extracts used previously in holoenzyme and Mediator purification (data not shown). In addition, the nuclear extract system is clearly Mediator-dependent and has been used extensively for biochemical study of transcription initiation, reinitiation, and activation (15, 16, 37).

The first step in Mediator isolation was affinity purification on anti-FLAG M2-agarose beads followed by peptide elution. Fig. 1 shows the small-scale affinity purification results from three yeast strains containing C-terminal FLAG-tagged Srb5, Srb6, and Rgr1, respectively. Recovery was measured by probing Western blots for Mediator subunits, pol II subunit (Rpb1), and several general transcription factors. Similar recoveries were obtained for all the strains: ~70% of Mediator subunits, 5–10% of pol II and <1% of both TFIIB and TFIIF were recovered. TFIIF and TBP were undetectable in the affinity-purified fractions (Table I). These results indicate that our FLAG-tag-based affinity purification is specific and efficient. A large-scale affinity-purified fraction from the FLAG-tagged Srb5 strain was then fractionated using gel filtration chromatography. In this step, we monitored each fraction by Western blotting using antibodies against Mediator subunits and general transcription factors (Fig. 2). The results revealed that the Mediator subunits existed in two major forms: one large complex (fractions 39–47) corresponding to an apparent size of ~1.9 MDa, and one smaller complex (fractions 63–69) corresponding to an apparent size of ~0.55 MDa. As shown below, the large complex is a form of pol II-Mediator complex (termed pol II-Med), and the small complex consists of a core subset of Mediator subunits termed Mediator core (Medc).

The molar amounts of factors in the gel filtration fractions were determined by comparison with recombinant protein standards for Srb2, Srb4, Srb10, Rpb3 (pol II subunit), TFIIB, and TFIIF (TFIIF subunit) (Fig. 2; data not shown; Table II). The pol II-Med complex principally contains pol II, and Mediator, including the Srb8–11 complex. As quantitated by Western blot of the pol II subunit Rpb3, pol II is estimated to be present at about one-half the molar ratio as compared with Srb2, -4, and -10. As shown below from comparison of the ratio of Mediator subunits to Rpb3 in preinitiation complexes (PICs), we believe that pol II is substoichiometric in the pol II-Med fractions compared with PICs. In contrast, a significantly lower amount of the general factors TFIIB and TFIIF was present in these fractions compared with Mediator subunits and pol II. We estimate that these factors are present at a level of 0.15–0.05 compared with other Mediator subunits. From these results, we conclude that the pol II-Med fractions contain primarily the pol II-Mediator complex, including Srb8–11 and a much lower amount of larger complexes containing TFIIB and/or TFIIF. In contrast to the holoenzyme complex isolated by Young and colleagues (18, 23), we do not detect TFIIF or SWI/SNF in these fractions (not shown).

In contrast to the pol II-Med fractions, the Medc fractions lack a subset of Mediator subunits (Fig. 2; Table II). Both the Gal11 and Srb10 Mediator subunits were missing from these fractions. In addition, Western blot assay of the Medc fraction with anti-Rgr1 and Sin4 showed the absence of these subunits (not shown). These results suggest the absence of both the Rgr1 and Srb8–11 modules. These modules have been implicated in transcription regulation but may not be essential for transcription, because most of these subunits are not encoded by essential genes. Therefore, the Medc fraction likely represents a core subcomplex of yeast Mediator. Probing of the gel filtration fractions for the Rpb3 subunit of pol II showed that a fraction of pol II dissociated from either the pol II-Med or Medc complexes during the purification and eluted at the position expected for pol II enzyme.

The gel filtration chromatography of affinity-purified Mediator was repeated using buffer containing 0.5 mM potassium acetate, compared with the results shown in Fig. 2 using 0.3 M...
potassium acetate (data not shown). Under these higher salt conditions, all of pol II dissociated from the Mediator complexes. However, the amount of the Medc fraction remained constant compared with the amount of intact Mediator. In addition, when gel filtration was performed using affinity-purified fractions derived from the Rgr1-FLAG strain, only the pol II-Med complex was detected (not shown). These results together suggest that the Medc complex is not derived from dissociation of the intact Mediator complex during purification but is rather a pre-existing complex in the nuclear extract. Most likely, the Mediator core subunits are in excess over other Mediator subunits in the extract.

To more fully characterize the composition of pol II-Med and Medc, we employed microcolumn HPLC-electrospray ionization tandem mass spectrometry (LC/MS/MS) (38) (Table III). This method uses liquid chromatography and tandem mass spectrometry to separate and fragment peptides in a complex mixture, thus offering the ability to identify >100 proteins in a single run without use of SDS-PAGE. Analysis of the pol II-Med fractions identified all 20 Mediator subunits, 9 of 12 pol II subunits, and 3 of 4 subunits of the Srb8–11 module (Table IIIA). For the Medc complex, we identified 13 Mediator subunits, 7 Mediator subunits (Rgr1, Gal11, Hrs1, Med2, Sin4, Rox3, and Nut1) not detected (Table IIIB). Specifically, the first five undetected subunits in Medc are all subunits of Rgr1 module, which was previously implicated in both positive and negative control of yeast transcription. Mass spectrometry analysis of the two fractions also identified ribosomal subunits as contaminants in the pol II-Med and Medc fractions. Specifically, many of the proteins of the large ribosomal subunit were identified in the pol II-Med fraction. Probing the FLAG affinity-purified fractions for TCM1 (RPL3), a protein in the large ribosomal subunit, indicated that <0.2% of total TCM1 in the starting extract contaminated the affinity-purified material (not shown). However, because ribosomal subunits are present at levels at least 500-fold over Mediator subunits (39), they represent a significant source of contamination. Probing of the gel filtration fractions with the TCM1 antisera showed near coelution of the large ribosomal subunit and pol II-Med, indicating the close similarity in molecular size of these two complexes (not shown).

Although the more sensitive Western blot assays showed detectable amounts of TFIIB and TFIIF in the pol II-Med fractions, these factors were not detectable in the mass spectrometry analysis, probably because they are quite substoichiometric compared with the other polypeptide subunits. A human homolog to the yeast Soh1 protein has been found in the thyroid hormone receptor-associated proteins-SRB/MED cofactor complex and PC2 Mediator complexes (3). However, yeast Soh1 was not found in either the pol II-Med or Medc complexes. It is possible that some subunits were not detected because they were substoichiometric or simply missed due to the complexity of the samples.

**In Vitro Transcription Assay of Mediator-containing Complexes**—To investigate the role of the two Mediator-containing complexes in transcription, in vitro transcription was performed using the activator Gal4-VP16 and the yeast HIS4 promoter. Transcription was assayed by complementation of the yeast HIS4 promoter. Transcription was assayed by complementation of the yeast HIS4 promoter. Transcription was assayed by complementation of the yeast HIS4 promoter.

**Composition Analysis of PICs and the pol II-Med Complex**—The results above show that the pol II-Med fraction is nearly...
Mediator complexes pol II Medc (10) and Srb10 homologs (Cdk8 and cyclin C) (13, 40, 41). Because our pol II-Med fraction was shown to be active in transcription, we tested whether Srb8–11 was recruited to PICs under these conditions. For this assay, we used the HIS4 promoter immobilized to magnetic beads (15, 16). In this system, PICs are formed on the immobilized template using nuclear extracts, washed, and then eluted from the beads by digestion of the DNA with PstI which cuts just upstream of the single Gal4 binding site at the promoter. Factors bound to the template were assayed by Western blotting. As shown in Fig. 4, both the ΔMed9 and ΔSrb2 extracts have a severe defect in recruitment of pol II, TFIIB, and Mediator subunits to the promoter compared with wild-type extracts (Fig. 4; compare lanes 1 and 4 with lanes 8 and 9). PIC formation was restored to wild-type levels by addition of the pol II-Med complex (lanes 2 and 6). Srb10 was clearly a component of the PICs formed in vitro both with wild-type extracts and extracts supplemented with the pol II-Med fraction.

When both the ΔMed9 and ΔSrb2 extracts were supplemented with the pol II-Med fraction, equivalent levels of Srb5-FLAG were seen in PICs compared with the levels seen when nuclear extracts made from the Srb5-flag strain were used to form PICs (compare lanes 2, 6, and 8). This suggests that the purified pol II-Med complex is stable when added to the extracts and that subunits do not exchange between this complex and other Mediator subunits preexisting in the extracts. This nonexchange likely explains why the Medc complex has low activity when added to nuclear extracts containing the remaining Mediator subunits.

To compare the relative amounts of subunits in the PICs to the ones in the purified pol II-Med fraction, we also analyzed the pol II-Med fraction in the same Western blot (Fig. 4, lane 7). This analysis shows that about 2-fold more Rpb3 was present on PICs than in the pol II-Med fraction. This suggests that pol II is somewhat unstable in the Mediator complex and dissociates upon fractionation by either affinity chromatography and/or gel filtration. In the complementation experiment, pol II-Med fractions containing pol II may be selectively recruited to the promoter, or the Mediator complex lacking pol II may associate with free pol II in the extract. In contrast to this behavior, Srb10 was substoichiometric in PICs compared with the pol II-Med complex (about a 2-fold difference between PICs and pol II-Med); (Fig. 4, compare lane 7 with lanes 2, 6, 8, 9). One possible explanation for this result is that the Srb8–11 module may be unstable in the PIC and a fraction of it dissociates upon washing and isolation of the PICs.

The yeast Mediator complex is critical for both basal and activated transcription in vitro using a crude transcription system. In vivo, Mediator is necessary for nearly all pol II transcription with only a few reported exceptions (42, 43). Related Mediator complexes have been found in mammalian cells where they may be central to integration of gene control signals. In both yeast and mammalian cells, Mediator com-

**Fig. 4.** Composition of PICs and the pol II-Med. PICs were formed using the Immobilized Template Method. PICs and the pol II-Med complex were resolved by SDS-PAGE and analyzed by Western blotting using antibodies directed against the proteins indicated. Nuclear extracts were made from ΔMed9, ΔSrb2, Srb5-FLAG, and wild-type (WT) strains. For each reaction, 180 μg of nuclear extract was incubated with immobilized templates for 40 min, along with recombinant rSrb2 (500 ng) or pol II-Med (2 μl) where indicated, to form PICs. The PICs were then isolated and analyzed as described under “Materials and Methods.” Lanes 1–6, 8, and 9, PICs formed on immobilized template; lane 3, nonspecific binding to the Dynabeads as a control; lane 7, 0.13 μl of pol II-Med complex was loaded on the same gel for comparison with PICs.

**Fig. 5.** Schematic diagrams of Mediator complexes pol II-Med (A) and Medc (B).
plexes have been isolated in multiple forms. Whether these multiple forms are pre-existing distinct complexes in vivo or whether they arise during purification is not known.

Here, we attempted to address the nature of the yeast Mediator-containing complexes using affinity purification and gel filtration chromatography, which avoids the high salt and ion exchange methods, used previously. As a starting material, we used yeast nuclear extracts, which have a higher specific activity in transcription than the previously used whole cell extracts. Using these methods, we found two major Mediator-containing complexes in nuclear extracts. The first complex, termed pol II-Med, migrates in gel filtration similarly to the large ribosomal subunit and contains principally pol II and Mediator, including the Srb8–11 module. This fraction also contains detectable amounts of TFIIB and TFIIF, but at levels 1/7 to 1/20 the levels of other Mediator subunits analyzed. Because the gel filtration cannot separate large complexes of this size differing by only a few polypeptides, we propose that this pol II-Med fraction contains mainly a complex of pol II and Mediator (Fig. 5). Additionally, a much smaller amount of this pol II contains detectable amounts of TFIIB and TFIIF, but at levels 1/7 to 1/20 the levels of other Mediator subunits analyzed.

This latter module was lost during their ion exchange purification. Using these methods, we found two major Mediator-containing complexes using affinity purification and gel filtration chromatography, which avoids the high salt and ion exchange methods, used previously. As a starting material, we used yeast nuclear extracts. We thank T. Reeves for comments on the manuscript.

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