Genome-associated RNA Polymerase II Includes the Dissociable Rpb4/7 Subcomplex

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Yeast RNA polymerase (Pol) II consists of a 10-subunit core enzyme and the Rpb4/7 subcomplex, which is dispensable for catalytic activity and dissociates in vitro. To investigate whether Rpb4/7 is an integral part of DNA-associated Pol II in vivo, we used chromatin immunoprecipitation coupled to high resolution tiling microarray analysis. We show that the genome-wide occupancy profiles for Rpb7 and the core subunit Rpb3 are essentially identical. Thus, the complete Pol II associates with DNA in vivo, consistent with functional roles of Rpb4/7 throughout the transcription cycle.

Pol II is the enzyme responsible for mRNA synthesis during transcription of protein-coding genes in eukaryotic cells. The structure of the complete 12-subunit Pol II is known and consists of a 10-subunit core enzyme, which includes the active center, and the peripheral heterodimer of subunits Rpb4 and Rpb7 (Rpb4/7 subcomplex) (1–4). Whereas Rpb7 is essential for viability of the yeast Saccharomyces cerevisiae, Rpb4 is not, but it becomes essential at temperature extremes (5). In vitro, yeast Rpb4/7 is required for transcription initiation, can dissociate from Pol II, and is dispensable for catalytic RNA elongation (6). Rpb4/7 binds single-stranded nucleic acids and mediates a step during initiation subsequent to promoter DNA binding (7). These observations are consistent with the idea that Rpb4/7 is present during initiation at promoters but then dissociates from Pol II.

However, evidence has accumulated for additional functional roles of Rpb4/7 during transcription. Rpb7 remains associated with early elongation complexes (8) and binds Nrd1, a protein involved in RNA 3′-end processing (9). Rpb4/7 can be cross-linked to the transcribed region, and loss of Rpb4 decreases the association with 3′-processing factors and alters usage of the polyadenylation site at a tested gene (10). Thus, recent data suggest that Rpb4/7 is an integral part of the Pol II enzyme and is required not only for initiation but also for 3′-RNA processing at the end of transcription.

To investigate whether Rpb4/7 generally associates with Pol II in vivo, we carried out chromatin immunoprecipitation in yeast coupled to tiling microarray analysis at a technical resolution of 32 base pairs. We demonstrate that the occupancy profiles of Rpb7 and the Pol II core subunit Rpb3 are virtually identical, showing that the complete Pol II, including Rpb4/7, associates with DNA genome-wide. These data are consistent with functional roles of Rpb4/7 throughout the transcription cycle.

EXPERIMENTAL PROCEDURES

Yeast Strains—Experiments were performed with S. cerevisiae strains W303 wild type (MATa, ura3-1, trp1-1, his3-11,15, leu2-3,112, ade2-1, can1-100, GAL+) and W303 carrying a tandem affinity purification (TAP) tag sequence fused to the C terminus of the gene encoding Rpb3 (MATa, ura3-1, his3-11,15, leu2-3,112, ade2-1, can1-100, GAL+, RPB3-TAP::TRP1). In addition, S. cerevisiae S288C strains RPB3-TAP and RPB7-TAP (ATCC 201388:MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0; Open Biosystems) were used.

Production of Monoclonal Antibodies against Rpb4/7—Lou/C rats were immunized subcutaneously and intraperitoneally with a mixture of 50 µg of purified recombinant Rpb4/7-His fusion protein (2), 5 nmol of CpG oligonucleotide (ODN 2006; TIB Molbiol, Berlin, Germany), 500 µl of phosphate-buffered saline, and 500 µl ofIncomplete Freund’s Adjuvant (IFA). After a six-week interval a final boost without adjuvant was given 3 days before fusion of the rat spleen cells with the murine myeloma cell line P3X63-Ag8-653. Hybridoma supernatants were tested in an enzyme-linked immunosorbsent assay using bacterial extracts from Escherichia coli expressing either the Rpb4/7 fusion protein or a His-tagged fusion protein. Antibodies B4 15H8 (rat IgG1) and B4 5F5 (rat IgG) recognized the protein specifically in Western blotting and were used for this study.

Chromatin Immunoprecipitation (ChIP)—ChIP was performed essentially as described (11). Briefly, chromatin was sheared with a BioruptorTM UCD-200 (Diagenode) using 25 × 30 s cycles with 30 s breaks at an output of 200 W. Before proceeding with immunoprecipitation experiments 20 µl of chromatin solution was put aside and marked an Input sample. For immunoprecipitation of TAP-tagged Rpb3 and Rpb7, IgG-Sepharose1 M 6 Fast Flow (GE Healthcare) was used. Precipita-
TABLE 1
Pearson correlation coefficients between occupancy profiles

| Comparison            | Profile 1 | Profile 2 | Pearson correlation |
|-----------------------|-----------|-----------|---------------------|
| Biological replicates  | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| Rpb3 vs. Rpb7         | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| Different strains*    | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| TAP tag vs. antibody* | Rpb7-TAP  | S288C     | Rpb4/7          |

*Profiles are single measurements with the ChIP DNA tagged with the Cy5 dye.

Results and Discussion

**Genome-wide ChiP of Pol II Subunits**—To investigate whether the complete Pol II is the form of the enzyme that associates with the yeast genome or whether the complete Pol II and the core Pol II associate with different regions of the genome, we used ChiP coupled to occupancy profiling with high resolution tiling microarrays (ChiP chip). We used yeast strains that contained TAP tags at the C termini of the integral core subunit Rpb3 and the essential Rpb4/7 subunit Rpb7. Both subunit C termini are exposed in the Pol II structure (4), suggesting that the tag would not interfere with Pol II function in vivo.

**Analysis and Quality of ChiP Chip Data**—To investigate the reproducibility of the ChiP chip data, we carried out three biological replicate experiments with the S288C Rpb3-TAP strain. The reproducibility of the obtained data were very high, resulting in Pearson correlations between individual profiles of around 0.9 (Table 1). The Pearson coefficient is 1 for a perfect correlation and 0 for uncorrelated signals. Similarly, two bio-

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Pearson correlation coefficients between occupancy profiles

| Comparison | Profile 1 | Profile 2 | Pearson correlation |
|-----------|-----------|-----------|---------------------|
| Biological replicates* | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| Rpb3 vs. Rpb7         | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| Different strains*    | Rpb3-TAP  | S288C     | Rpb3-TAP            |
| TAP tag vs. antibody* | Rpb7-TAP  | S288C     | Rpb4/7          |

*Profiles are single measurements with the ChIP DNA tagged with the Cy5 dye.
logical replicates of S288C Rpb7-TAP profiles were obtained and showed very good agreement (Pearson correlation, 0.90; Table 1). Noise could be dramatically reduced using dual color microarray technology and replica measurements with interchanged dyes (13) (Fig. 1, A–C, and Table 1).

**Occupancy Profiles for Rpb3 and Rpb7 Are Essentially Identical**—We next compared the replica-averaged, noise-reduced profiles for Rpb3 and Rpb7. Differences in the two profiles would indicate that part of the genome associates with the complete Pol II and part of the genome associates with the core enzyme, and thus Rpb4/7 is dissociable in vivo. In contrast, a high similarity of the two profiles would indicate that Rpb4/7 is always part of the DNA-associated Pol II in vivo.

Comparison of the average, noise-reduced occupancy profiles for Rpb3-TAP and Rpb7-TAP showed that they are highly correlated throughout the genome (Fig. 1, B and C). There are only small differences between the profiles (Fig. 1E and supplemental Fig. S2). Weak systematic deviations from zero occur...
only for a small fraction of genomic locations with the highest signal intensity (supplemental Fig. S2; see discussion below). A Pearson correlation coefficient of 0.91 between the averaged Rpb3 and Rpb7 profiles compares favorably with the correlation obtained for biological replicates of the same profile (0.88; Table 1). Thus, statistically the agreement between the two average profiles of Rpb3 and Rpb7 was as good as the agreement between individual replicate profiles of the same strain. The near identity of the two profiles, within experimental errors, indicates that Rpb4/7 is present in DNA-associated Pol II genome-wide.

The Profiles Are Not Systematically Influenced by the Type of Yeast Strain—We next investigated whether the obtained occupancy profiles change when a different strain of yeast is used. We compared the Rpb3-TAP profile obtained with yeast strain S288C to a profile obtained under identical conditions with a W303 strain. The obtained profiles were highly similar, resulting in a Pearson correlation of 0.90 (Table 1), showing that the type of yeast strain did not influence the results.

The Profiles Are Not Influenced by the Affinity Tag—It is possible that the cellular function of Rpb7 is perturbed by its fusion to the TAP tag. To investigate this, we purified the recombinant stoichiometric Rpb4/7 subcomplex as described (2) and raised monoclonal antibodies against the pure subcomplex (“Experimental Procedures”). We then used this monoclonal antibody to measure an unbiased occupancy profile for Rpb4/7. The resulting occupancy profile contained more noise than the profiles obtained from TAP-tagged strains, but it did not show any systematic disagreement with the profile obtained from TAP-tagged strains (Fig. 1D), resulting in a Pearson correlation of 0.72 (0.79 for the smoothed traces; Table 1). Thus, the profiles obtained from TAP-tagged strains reflect those of unperturbed yeast cells and were not considerably influenced by the presence of the TAP tag.

Correlation of the Pol II Occupancy Profiles with Genome Features—The ChIP chip data contain a lot of information beyond the observation that Rpb4/7 is a component of DNA-associated Pol II in vivo. Analyses of Pol II occupancy profiles over a part of the yeast genome or at lower resolution were presented recently (14–16), but a comparison of these published profiles with our data is hampered because of the use of different experimental conditions and technological platforms.

To analyze which genomic features correlate with Rpb3 occupancy, we identified all genomic regions with a ChIP chip signal above an occupancy threshold that was varied continuously (Fig. 2A). We added up the base pairs within these regions belonging to the genomic features annotated in the Saccharomyces Genome Data base (version 11, March 2008). The colored areas in Fig. 2A indicate the fractions of the regions annotated by various genomic features at a chosen Pol II threshold. A threshold of zero corresponds to equal fluorescent signals from Rpb3 and the genomic background. The solid line shows the proportion of the genome above the threshold. Fig. 2B shows how much certain genomic features are overrepresented in regions with Pol II occupancy above a given threshold.

Pol II Distribution over the Genome—This analysis provides general insights into the global distribution of Pol II in growing yeast cells. First, most Pol II is bound to highly transcribed genes that encode small nuclear RNAs, small nucleolar RNAs, or ribosomal proteins (Fig. 2). Second, genomic repeat regions that are expected to be transcriptionally silenced show very low Pol II occupancy. Third, Pol III-transcribed genes that encode tRNAs do not show Pol II enrichment. Fourth, the fraction of all genes and promoter regions is approximately constant over a large range of Pol II occupancy thresholds (Fig. 2A). This supports previous reports that have found no evidence for widespread accumulation of Pol II by pausing at promoters in yeast, in contrast to higher eukaryotes (17). However, our findings are
also consistent with other ChIP chip studies showing the preferential location of Pol II on promoter-bearing intragenic regions in yeast cells in stationary phase (15) because our data were obtained from yeast cells harvested in their logarithmic growth phase.

**Persistent Presence of Rpb4/7**—After this manuscript was submitted, a study comparing the genome-wide distribution of core Pol II and Rpb4/7 subcomplex was published (18). This study showed at lower resolution that Rpb3 and Rpb4 profiles are similar and generally agrees with our findings. The authors also note that Rpb4 can be underrepresented with respect to Rpb3, an effect that is more pronounced for shorter genes. However, we could not confirm that this is a significant or systematic effect, as demonstrated below.

The ratio of Rpb7 to Rpb3 does not fall below 0.78, and such deviations from 1 are limited to a very small fraction of the genome (Fig. 2C). We investigated whether these minor local deviations show systematic features and thus may be biologically significant. We analyzed the Rpb7-Rpb3 difference signal separately for several genomic categories, including ribosomal protein genes, nonribosomal protein genes, and snoRNA (Fig. 2C). This revealed that the difference signal increases with increasing occupancy on the x axis. The higher occupancy of Rpb3 versus Rpb7 for ribosomal proteins can be explained by the generally higher total Pol II occupancy on ribosomal protein genes. Rpb7 occupancy also did not depend on the position along the gene. The promoter regions and 5′ ends of genes show essentially the same difference signal as the coding regions. Thus, the ratio of Rpb3 to Rpb7 occupancy neither depends on the genomic feature type nor on the position along genes. Thus, there is no evidence for a functional significance of the minor local deviations between Rpb3 and Rpb7 levels.

**Functional Roles of Rpb4/7**—Our data suggest that Rpb4/7 is generally required throughout the transcription cycle in vivo and argue against models that propose the dissociation of Rpb4/7 during transcription. Our results are consistent with functional roles of Rpb4/7 during initiation (6, 7) as well as 3′-RNA processing (10). They are also consistent with microarray-based gene expression analysis of yeast strains lacking the gene for Rpb4, which suggested that Rpb4 is globally required for Pol II transcription (19, 20). They are further consistent with the observation that Rpb4 is essential in the fission yeast *Schizosaccharomyces pombe* and that Rpb4/7 is present in *S. pombe* Pol II in stoichiometric amounts during exponential cell growth (21).

Finally, our results are not inconsistent with the recent observations that Rpb4/7 shuttles between the nucleus and cytoplasm (22) and that Rpb4 and Rpb7 play roles in P-body function and mRNA decay (23), because it is possible that Rpb4/7 dissociates from core Pol II during transcription termination and that there is a cellular pool of free Rpb4/7. It also remains possible that Rpb4/7 dissociates under certain growth conditions.

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