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Genomic Binding Profiles of Functionally Distinct RNA Polymerase III Transcription Complexes in Human Cells

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Abstract

Genome-wide occupancy profiles of five components of the RNA Polymerase III (Pol III) machinery in human cells identified the expected tRNA and non-coding RNA targets and revealed many additional Pol III-associated loci, mostly near SINEs. Several genes are targets of an alternative TFIIB containing Brf2 instead of Brf1 and have extremely low levels of TFIIC. Strikingly, expressed Pol III genes, unlike non-expressed Pol III genes, are situated in regions with a pattern of histone modifications associated with functional Pol II promoters. TFIIC alone associates with numerous *ETC* loci, via the B box or a novel motif. *ETCs* are often near CTCF binding sites, suggesting a potential role in chromosome organization. Our results suggest that human Pol III complexes associate preferentially with regions near functional Pol II promoters and that TFIIC-mediated recruitment of TFIIB is regulated in a locus-specific manner.

RNA Polymerase III (Pol III) is responsible for the synthesis of tRNAs and other non-protein-coding RNAs (ncRNAs) in eukaryotes^{1–3}. Metazoan Pol III genes are classified into three types, each of which is transcribed by Pol III in concert with other multi-subunit general transcription factors. At tRNAs and other Pol III genes categorized as type 2, the recognition factor, TFIIC, binds to DNA sequences termed A and B boxes, which are typically contained entirely within the body of the structural gene. TFIIC recruits the initiation factor, TFIIB, which consists of the TATA-binding protein (TBP), Bdp1, and Brf1 subunits. These proteins recruit the polymerase to begin transcription. At the type 1

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Accession codes

The DNA sequencing datasets described in this work are available at <http://genome.ucsc.edu/ENCODE/>.

gene encoding the 5S rRNA, transcription involves a dedicated transcription factor, TFIIA, which recognizes special elements within the gene and recruits TFIIC, TFIIB, and Pol III.

Other Pol III-transcribed genes termed type 3 have upstream, gene-external promoters, much like Pol II-transcribed genes. At these loci, a proximal sequence element (PSE) is bound by the SNAPc complex, which is involved in transcription of small nuclear RNAs by either Pol II or Pol III. At Pol III promoters bound by SNAPc, TATA-bound TBP specifies Pol III transcription by recruiting the Brf2 subunit into an alternative TFIIB comprising TBP, Bdp1, and Brf2, followed by Pol III recruitment¹⁻³. *In vitro*, Brf2-dependent Pol III genes can be transcribed in the absence of Brf1 or TFIIC³.

Whole-genome chromatin immunoprecipitation studies using microarrays have been performed on subunits of each of these general transcription factors in yeast. In *S. cerevisiae*, complete Pol III complexes are found at tRNA genes and at loci encoding a variety of ncRNAs⁴⁻⁶. At these regions, the general transcription factors TFIIC, TFIIB, and Pol III are present at a fairly constant ratio, suggesting that the amount of TFIIC binding to promoters is predictive of the level of functional Pol III transcription complexes *in vivo*. However, while TFIIC-mediated recruitment of TFIIB shows little, if any, promoter specificity, TFIIB recruitment is generally repressed by Maf1 in response to environmental signals⁷⁻⁹.

In contrast to conventional Pol III-transcribed genes, eight loci with incomplete transcription complexes consisting only of TFIIC were identified in *S. cerevisiae* and termed *ETC*, for “Extra TFIIC”⁵. *ETC* loci are characterized by a sequence motif that contains the B box and an additional three conserved nucleotides, suggesting that TFIIC’s association with this special motif influences its potential to recruit the remainder of the Pol III machinery. The *ETC* loci are remarkably well-conserved both structurally and functionally across closely related yeast species⁵, indicating that these regions are biologically important. Similar loci were later identified in *S. pombe*, where they are more numerous and named *COCs* (“chromosome organizing clamps”) due to their apparent role in higher-order chromosome organization¹⁰. It is unknown whether *ETC* loci are present in metazoans.

Here, by analyzing subunits of the three major Pol III transcription factors, we examine the occupancy profile of the Pol III transcription machinery on a genome-wide scale. We identify new Pol III targets, distinct classes of *ETC* loci, and distinct binding profiles of Brf1- and Brf2-containing isoforms of TFIIB. Further, we find binding of the Pol III machinery to target promoters to be strongly linked with (and perhaps dependent on) a nearby functional Pol II promoter and associated histone modifications.

RESULTS

Genome-wide occupancy of five Pol III transcription factors

We performed chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) on three independent biological replicates of K562 cells, using antibodies against five proteins involved in RNA polymerase III transcription. These antibodies correspond to subunits of the promoter recognition factor TFIIC (TFIIC-110 subunit), the initiation

factor, TFIIB (Bdp1 and Brf1 subunits), and the polymerase itself (Rpc155 subunit). In addition, we used an antibody against Brf2, which is involved in the transcription of Pol III-transcribed genes with gene-external promoter architecture.

The overall quality and reproducibility of the data were high, with correlation coefficients between replicates typically ranging between 0.79 and 0.95. (Supplementary Fig. 1). The Brf2 data were lower in overall quality and reproducibility (correlation coefficients between 0.41 and 0.68), but previously known Brf2-bound loci were easily identifiable using high-stringency thresholds. Peak lists for the five proteins are shown in Supplementary Fig. 2.

Expected and novel targets of RNA polymerase III

The Rpc155 antibody yielded by far the most robust ChIP signals of the antibodies. Using a stringent threshold, we identified 1520 Pol III targets, which we then used as the reference list when comparing occupancies by the Pol III transcription factors. To determine the occupancy levels of all five proteins at each Rpc155-associated locus, we counted the sequence reads (“tags”) for every protein within a 300 bp window centered on the coordinate with maximal Rpc155 occupancy. By determining the relative occupancy of Pol III factors at each genomic region, we obtained information about the nature of the transcription complexes at the Pol III promoters in a manner analogous to that used for Pol II preinitiation complexes¹¹.

The general transcription factors show a high degree of co-occupancy at Pol III loci (Fig. 1A–D). The TFIIB components Brf1 and Bdp1 were each highly correlated ($R^2 = 0.77$ and 0.80) with Rpc155 at Pol III loci, validating our Rpc155-associated loci as meaningful targets of the complete Pol III machinery. Fittingly for two subunits of the same factor, Bdp1 and Brf1 showed a correlation ($R^2 = 0.89$) comparable to that between replicate IPs of the same protein. At Rpc155 targets, TFIIC was markedly less well correlated ($R^2 = 0.56$) with polymerase than was TFIIB. As will be discussed further below, this suggests that the degree of TFIIC occupancy is not strictly related to the level of Pol III transcription.

We plotted the relative position of each protein with respect to transcription start sites (TSS) of Pol III genes (Fig. 1E and F). The peak of Rpc155 covers a region spanning from 5' of the TSS into the structural gene. TFIIC occupancy peaks downstream of the TSS and within the gene, reflecting the gene-internal position of the DNA sequences recognized by this factor. The TFIIB components Brf1, Brf2, and Bdp1 have occupancy peaks upstream of the TSS, in accord with their role in initiation. Thus, the mapping afforded by the sequencing data is of sufficient resolution to distinguish closely spaced binding sites even within small Pol III genes.

As expected¹, the complete Pol III machinery is found at genes encoding tRNAs, 5S rRNA, U6, hY, 7SK RNA, and RNase P RNA (Fig. 2A), representing all three types of Pol III promoters. In general, TFIIC, TFIIB, and Pol III association with these loci is robust (> 100-fold enrichment of Pol III in many cases). Of the 513 tRNA genes, 392 are targets of Rpc155 by our stringent peak-calling criteria. In addition, we detected Pol III occupancy at 41 out of the 172 tRNA pseudogenes in the human genome. Because of the clustering of many tRNA genes, some Rpc155 peaks contain more than one tRNA target. The percentage

of expressed genes for each type of tRNA is variable, with, for example, almost all cysteine tRNA genes, but fewer than half of glutamic acid tRNA genes, being occupied by the polymerase (Fig. 2B).

In addition to the expected targets, many additional loci are associated with Pol III (Supplementary Fig. 3). These include NF90-associated RNAs encoded by *snaR* loci, which possess B boxes and Pol III termination sequences and are transcribed by Pol III *in vitro*¹². We do not observe Pol III at any annotated miRNA genes (except for *miR-1975* and *miR-886*, which are unlikely to be relevant, as they overlap completely with hY5 and vault RNAs that are known Pol III targets); this includes miRNA genes clustered on chromosome 19 that have been the subject of conflicting reports^{13,14}. Small but reproducible amounts of Pol III are associated with *U91*, which overlaps the ncRNA *SCARNA18*, and with *U13* snoRNA. A striking majority (90%) of the otherwise non-annotated Pol III-associated loci are near SINE elements. In general, these SINE-linked loci have considerably lower levels of Pol III association than observed at tRNA genes.

Active Pol III genes are near Pol II-dependent histone marks

In this paper, we define expressed and non-expressed tRNAs by virtue of Pol III association. While it is formally possible that Pol III association might not result in RNA synthesis, this is unlikely because tRNA levels are very high. We analyzed the proximity of expressed (i.e., Pol III-occupied) and non-expressed tRNAs with regard to nearby histone modifications. There is a marked dip in all histone marks corresponding exactly to the TSS of expressed tRNAs, suggesting a strong and localized nucleosome depletion over the TSS (Fig. 2C, solid lines). This apparent nucleosome depletion is similar to that observed at tRNA genes in *S. cerevisiae*^{15,16}, and it is likely due to nucleosome exclusion by the extremely high levels of transcription at Pol III genes.

Although Pol III peaks are often in proximity to Pol II peaks¹⁷, the functional relationship of this observation is unclear. Strikingly, for the 392 expressed Pol III genes, there is a high correlation with a pattern of histone marks typical of Pol II TSS regions and with occupancy by Pol II itself. This proximity is directional; Pol II occupancy peaks 5' of expressed Pol III genes. In contrast, no such pattern is observed for the non-expressed subset of tRNA genes (Fig. 2C, dashed lines). Together, these results suggest that the histone modification pattern at Pol II TSS regions is an important determinant of Pol III expression.

Brf2 sites lack Brf1 and are very low in TFIIC

Brf2 occupies U6 and several other loci with TATA-containing promoters located upstream of the sequences encoding mature RNA. *In vitro*, Brf2 is recruited by TBP in a TFIIC-independent manner to these promoters, forming a complex containing Bdp1 but lacking Brf1³. In accord with this biochemical observation, Brf1 and Brf2 occupancies are uncorrelated at Pol III-occupied loci (Fig. 3A). Furthermore, Brf1 occupancy at Brf2 sites is very low or absent (Fig. 3B), indicating that Brf1 is not required for transcription of Brf2-dependent genes *in vivo*. TFIIC association is also very low at Brf2 targets, although not entirely absent (~2% the level observed at Brf1-associated loci), suggesting that TFIIC is unnecessary for Pol III recruitment to Brf2 loci *in vivo* (Fig. 3B). Notably, the Brf2-occupied

locus with the highest TFIIC binding (though still only a small fraction of the TFIIC level at Brf1 targets), is *tRNA^{Sec}*, which in *X. laevis* uses both a gene-internal B box and the gene-external promoter structure typical of type 3 genes¹⁸.

Numerous *ETC* loci in human cells

TFIIC peaks vastly outnumber the peaks for the other transcription factors; our TFIIC target list consists of 5474 loci, in contrast with 1520 at Rpc155, even though fold-enrichments and hence assay sensitivity are higher for Rpc155. Unlike TFIIB loci, TFIIC targets are highly variable in the extent to which they are occupied by the other components of the Pol III machinery (Fig. 4A), with only a limited correlation ($R^2 = 0.37$) between Rpc155 and TFIIC at TFIIC targets. To identify human *ETC* loci, we first restricted our TFIIC peak list to an even higher level of stringency (see methods). As the median ratio of TFIIC to Rpc155 at tRNA genes is 0.06, we defined an *ETC* locus as having a TFIIC/Rpc155 ratio higher than 2.04, three standard deviations above this median ratio. *ETCs* by this definition do not need to be completely lacking in Pol III occupancy. We validated binding of Rpc155 and TFIIC at several normal and *ETC* targets using quantitative PCR analysis in real time (Fig. 4B). Under these strict criteria (only 3 tRNAs pass), we identify 1865 *ETCs* (listed in Supplementary Fig. 4) and suspect there are several thousand in the genome.

ETCs are often near Pol II genes and CTCF-bound regions

The distribution of TFIIC-occupied loci of both the *ETC* and the non-*ETC* types reveals a positional bias toward the transcription start sites of Pol II genes (Fig. 4C). *ETC* loci show a wide dynamic range in the amount of TFIIC binding, with some sites emerging as especially pronounced *ETCs*. The 181 *ETC* loci with the highest levels of TFIIC occupancy (>50 sequence reads per site) are strikingly well-correlated with the TSS of Pol II genes, with 68% being located within 1kb of a Pol II TSS (Fig. 4D). This is reminiscent of the *S. cerevisiae* *ETCs*, which are ~200–300 bp upstream of a neighboring Pol II transcribed gene. GO categories of Pol II genes adjacent to *ETC* loci show significant overrepresentation of nuclear-localized proteins. In addition, *ETCs* are significantly overrepresented in regions upstream of closely spaced, divergently transcribed pairs of Pol II genes. Of the 1431 shared upstream sequences for Pol II genes (defined as having TSS less than 1kb apart), 86 harbor *ETC* loci ($P < 10^{-300}$).

As expected from the proximity of strong *ETCs* to mRNA initiation sites, the *ETC* population shows histone modifications associated with functional Pol II promoters, with highest levels of these modifications near the TFIIC peaks (Fig. 4E). This pattern of histone modifications at *ETC* loci (dashed lines) differs from the pattern at expressed Pol III genes (solid lines), in which histone modifications appear very low near the promoter due to nucleosome loss accompanying high levels of Pol III transcription. Lastly, in accord with the observation that TFIIC targets in yeast play a role in genome organization^{10,19,20} and act as transcriptional insulator elements^{21–23}, TFIIC association at *ETC* loci is highly correlated with association of CTCF (Fig. 4F), a protein that interacts with cohesins and is involved in insulation, looping, and chromosome conformation^{24,25}. This observation suggests that TFIIC may play a role in chromosome organization in humans.

A novel sequence motif at a subset of *ETC* loci

De novo motif searching revealed that in contrast with tRNAs, which have A and B boxes, the *ETC* loci are characterized by one of two significantly overrepresented sequence motifs: the standard B box or a novel motif that may be loosely related to a motif for Ets transcription factors (Fig. 5A and B). The *ETC* motif coincides with the summit of TFIIC peaks at many *ETCs* (Fig. 5C). *ETC* and B box motifs are mostly mutually exclusive, with only 4 *ETC* loci having both motifs (Fig. 5D). The existence of an *ETC*-specific motif is analogous to the situation in *S. cerevisiae*, where *ETC* loci possess a novel motif extending the canonical B box. In both organisms, TFIIC could associate with these *ETC*-specific motifs in a manner structurally un conducive to the assembly of complete Pol III complexes.

ETC loci show cell-type specificity

To determine whether Pol III occupancy varies with cell type, we examined the genome-wide association of Pol III factors in a second cell line, HeLa S3. Interestingly, while binding of Pol III factors at tRNA loci is highly correlated between cell lines (Fig. 6A), there are differences in TFIIC occupancy at *ETC* loci between HeLa S3 and K562 cells (Fig. 6B). This observation suggests that TFIIC binding and/or TFIIB recruitment at *ETC* loci, but not tRNA genes, is influenced by cell-type-specific factors.

DISCUSSION

Genome-wide profiling reveals ~1500 Pol III-associated loci in K562 cells. In general, the expected 400–500 Pol III-transcribed genes encoding tRNAs and ncRNAs show very high levels of all three Pol III factors. In contrast, ~1000 Pol III-associated loci have not been previously described, and the vast majority of these (90%) are located near SINEs. The SINE-associated loci show much lower levels of Pol III factors as compared to tRNA genes, although these levels are clearly above the background. The transcriptional products of and biological functions of these Pol III-associated loci near SINEs are unknown. The prevalence of SINEs near these Pol III-associated loci might reflect DNA sequences resembling B or A blocks in a subset of SINEs.

Most, but not all, tRNA genes are occupied by the complete Pol III transcription machinery, and Pol III association at expressed vs. non-expressed genes differs by a factor of 100. Strikingly, the genomic regions in the vicinity of expressed and non-expressed Pol III genes are different. Expressed Pol III genes are located close to regions that have histone modifications characteristic of functional Pol II promoters and Pol II itself. In contrast, this distinctive chromatin signature is absent from non-expressed Pol III genes. These observations suggest that Pol III factors bind preferentially to genomic regions with a histone modification pattern generated by a functional Pol II promoter. We think it unlikely that non-expressed Pol III genes have defective TFIIC recognition sites, because they generally possess high-quality B box sequences. Conversely, TFIIC associates with fewer than 2% of the B box motifs in the human genome, indicating that the presence of a B box alone is insufficient for binding *in vivo*. In addition, the fact that *ETC* loci are located near Pol II promoters and associated histone modification strongly argues that TFIIC association depends on Pol II-generated chromatin regions.

The characteristic pattern of histone modifications at Pol II promoters depends on Pol II preinitiation complexes but not on extensive elongation, because Pol II is often paused just downstream of many promoters in a manner that precludes any appreciable transcription^{26,27}. This suggests that TFIIC binding to regions near functional Pol II promoters is largely, and perhaps completely, independent of Pol II transcriptional activity (i.e. mRNA synthesis). In accord with this suggestion, treatment of human cells with alpha-amanitin, an inhibitor that blocks Pol II transcription after preinitiation complex formation, has limited effects on Pol III transcription¹⁷. For these reasons, we speculate that TFIIC binding is strongly enhanced by the chromatin structure generated by nearby functional Pol II promoters. In principle, TFIIC binding might be increased by promoter accessibility due to histone acetylation reducing histone-DNA contacts, direct interactions with modified histones via an effector domain(s) in a TFIIC subunit or associated protein, or a histone variant (e.g. H2AZ) near Pol II promoter regions.

Accessibility to the recognition factor TFIIC is not, however, the only determinant of Pol III association in human cells. In *S. cerevisiae*, TFIIC levels are highly correlated with Pol III occupancy, and recruitment of the complete Pol III machinery exhibits a one-to-one correspondence with TFIIC binding. In contrast, the human TFIIC/Pol III ratio is considerably more variable than it is in yeast, and a wide range of TFIIC levels may recruit a given amount of polymerase. The presence of a Brf2 mechanism for Pol III recruitment in human cells is one obvious alternative pathway, allowing transcription with little or no TFIIC. At the opposite extreme are the *ETC* loci, which recruit little or no polymerase despite having high occupancy by TFIIC. Even at standard type 2 Pol III genes, the ratio of TFIIC to polymerase varies over a wide range, in contrast to the TFIIB/Pol III ratio, which is relatively consistent.

The different patterns of histone marks near *ETC*-type and transcriptionally active TFIIC sites suggest that TFIIC's ability to recruit TFIIB to type 2 genes might depend on a particular histone modification pattern. Indeed, Myc-dependent activation of Pol III transcription is associated with targeted histone acetylation and increased association of TFIIB²⁸. In a similar vein, transcription of type 3 genes is influenced by the CHD8 chromatin modifying protein²⁹. Interestingly, TFIIC either possesses histone acetylase activity^{30,31} and/or recruits the p300 histone acetylase³², such that it could actively participate in generating a chromatin state appropriate for TFIIB recruitment. Association of the initiator TFIIB is an important rate-limiting step in Pol III gene expression in humans, because the excellent correlation of TFIIB and polymerase levels suggests that Pol III occupancy follows linearly from TFIIB recruitment. In this regard, TFIIB is a target of regulation Maf1³³, Rb³⁴, p53³⁵, Erk³⁶, and Myc²⁸.

The presence of TFIIC at many loci without TFIIB or Pol III, much like similar loci in yeast, suggests a role for human TFIIC beyond its function in Pol III transcription. TFIIC bound to the intergenic region of closely spaced, divergently transcribed Pol II genes might be important for the regulation and separate expression of the two genes. In yeast, TFIIC-bound loci function as heterochromatin barriers and insulators^{10,21,22} and also participate in higher-order chromosome organization^{10,37}. The correlation of TFIIC peaks with CTCF is

noteworthy and suggests the possible involvement of human TFIIC in chromosome organization.

METHODS

Cell culture and formaldehyde crosslinking

We cultured three separate preparations of K562 cells in RPMI supplemented with 10% (v/v) FBS to a density of 2×10^7 cells/ml and crosslinked the cells with 1% (v/v) formaldehyde for 10 minutes before harvesting and freezing them.

Chromatin immunoprecipitation and quantitative PCR

We performed chromatin preparation and chromatin immunoprecipitations as previously described³⁸ on material derived from three independent biological replicates of 2×10^7 cells each. The antibodies used were 2663 for Bdp1³⁹, 128 for Brf1⁴⁰, 4286 for TFIIC110³⁴, and 1900 for Rpc155³⁹. Antibody 4295 against Brf2 was raised by immunizing rabbits with keyhole limpet hemocyanin coupled to synthetic peptides VSRSQQRGLRRVRDLC and SDSEIEQYL RTPQEV, corresponding to human Brf2 residues 66–80 and 385–400, respectively. 2/3 of each IP was used for Illumina sequencing. We performed quantitative PCR in real time as previously described⁵. We obtained occupancy values for genomic loci by subtracting the value obtained for the control H3 locus and then expressing the results relative to the level at the arginine tRNA locus on chromosome VI that was arbitrarily defined as 100.

Illumina sequencing

We subjected input control and IP DNA to amplification and sequencing on an Illumina Genome Analyzer as previously described⁴¹. The IP DNA from each replicate was amplified separately and sequenced in different lanes, yielding an average read count of more than 15–19 million.

Data analysis and peak identification

We analyzed only those matches aligning to unique positions in the genome. For most factors, we obtained 9–10 million uniquely aligned reads per K562 replicate and 14–15 million per HeLa replicate. TFIIC-110 gave a lower percentage of uniquely mappable reads (approximately 4 million per replicate), most likely because TFIIC recognizes a DNA region with a high degree of sequence identity across tRNAs. We used the MACS peak-finding software⁴² to analyze the sequencing data and identify targets of each proteins. For each factor, sequence data from the three replicates were analyzed both individually and also as a single merged data set. We considered a peak to be a target if it had a pValue in the combined data set of 1×10^{-7} or better, with the additional requirement that two or more replicates individually yield pValues of 1×10^{-5} or lower. When looking for *ETCs*, we increased the stringency of the cutoff used to define TFIIC sites by requiring that a TFIIC peak be defined by an overall pValue of 1×10^{-7} or better and a pValue of 1×10^{-5} or better in all three replicates. We visualized peaks using the Affymetrix Integrated Genome Browser. We obtained genome information from the UCSC Genome Browser and the Eddy lab tRNA database. Data for histone modifications and CTCF came from the Broad Institute

database, and Pol II ChIP-sequencing data was from the Yale ENCODE sequencing project data on the UCSC genome database. We identified sequence motifs using the MEME suite⁴³.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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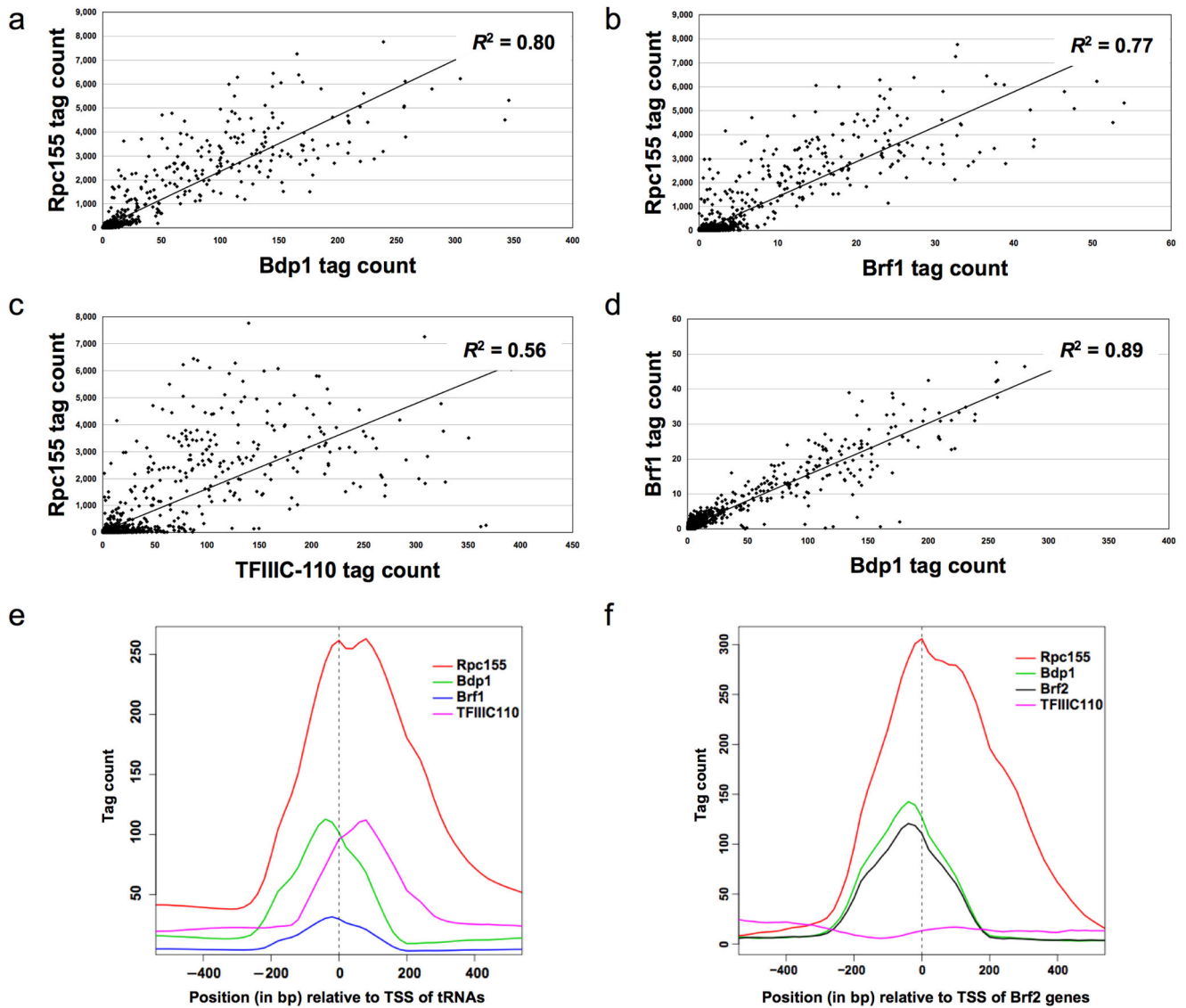


Figure 1.

Pairwise comparison of occupancy by proteins at Pol III loci. Each plot compares the average number of sequence reads (“tag count”) for the indicated proteins within the 300bp window centered at each Rpc155 summit. (a) Rpc155 vs Bdp1. (b) Rpc155 vs Brf1. (c) Rpc155 vs TFIIC-110. (d) Brf1 vs Bdp1. (e) Distribution of Bdp1, Brf1, TFIIC-110, and Rpc155 association with respect to transcription start sites (TSS) of tRNAs. (f) Distribution of Bdp1, Brf2, TFIIC-110, and Rpc155 association with respect to TSS of Brf2-associated genes.

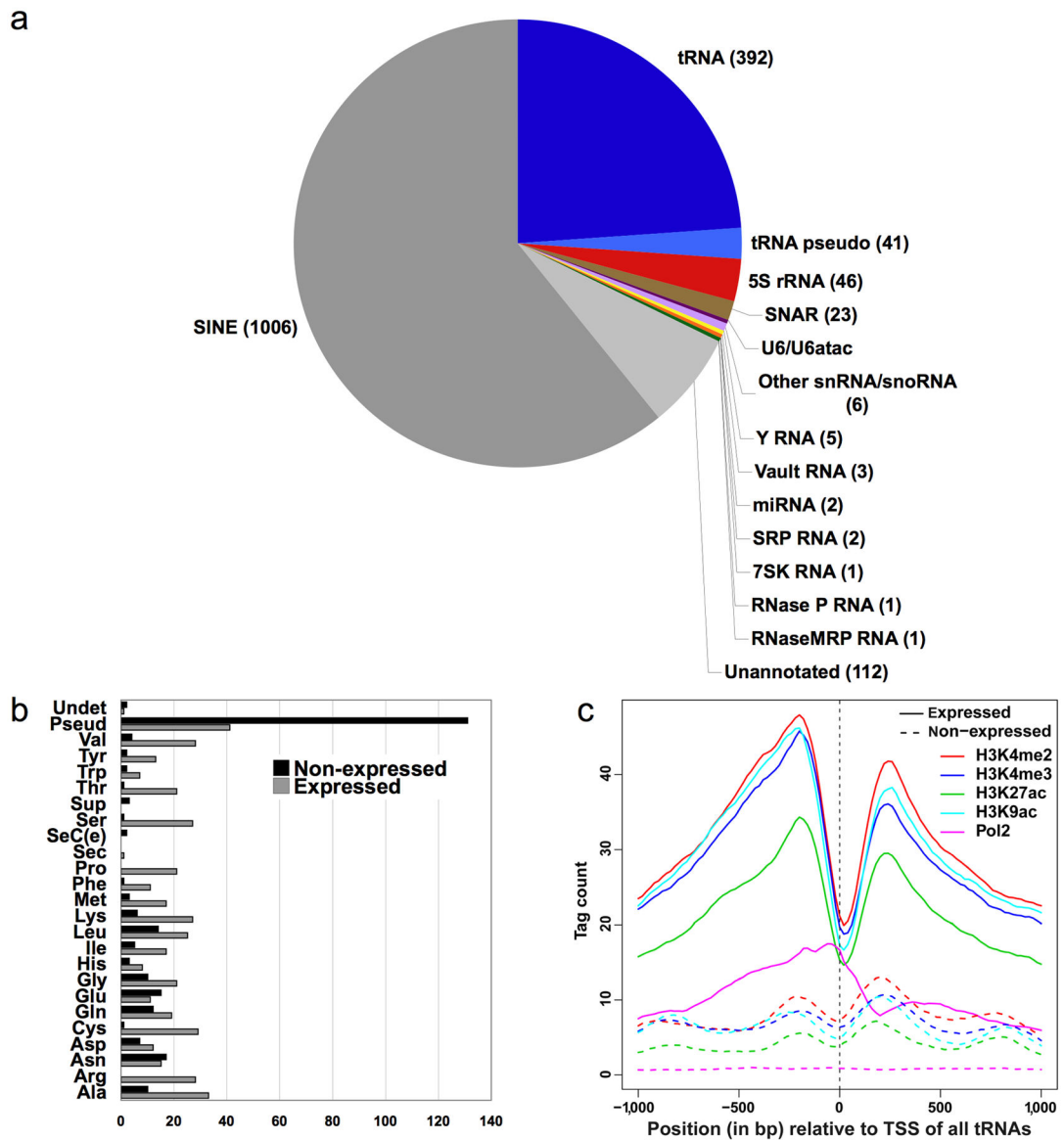


Figure 2. Properties of Pol III targets. **(a)** Pie chart depicting the categories of Pol III-occupied loci. **(b)** Occupancy of tRNA genes by isotype. **(c)** Positions of histone modifications and Pol II occupancy relative to expressed (i.e., Pol III-occupied, solid lines) or non-expressed (dashed lines) tRNA genes.

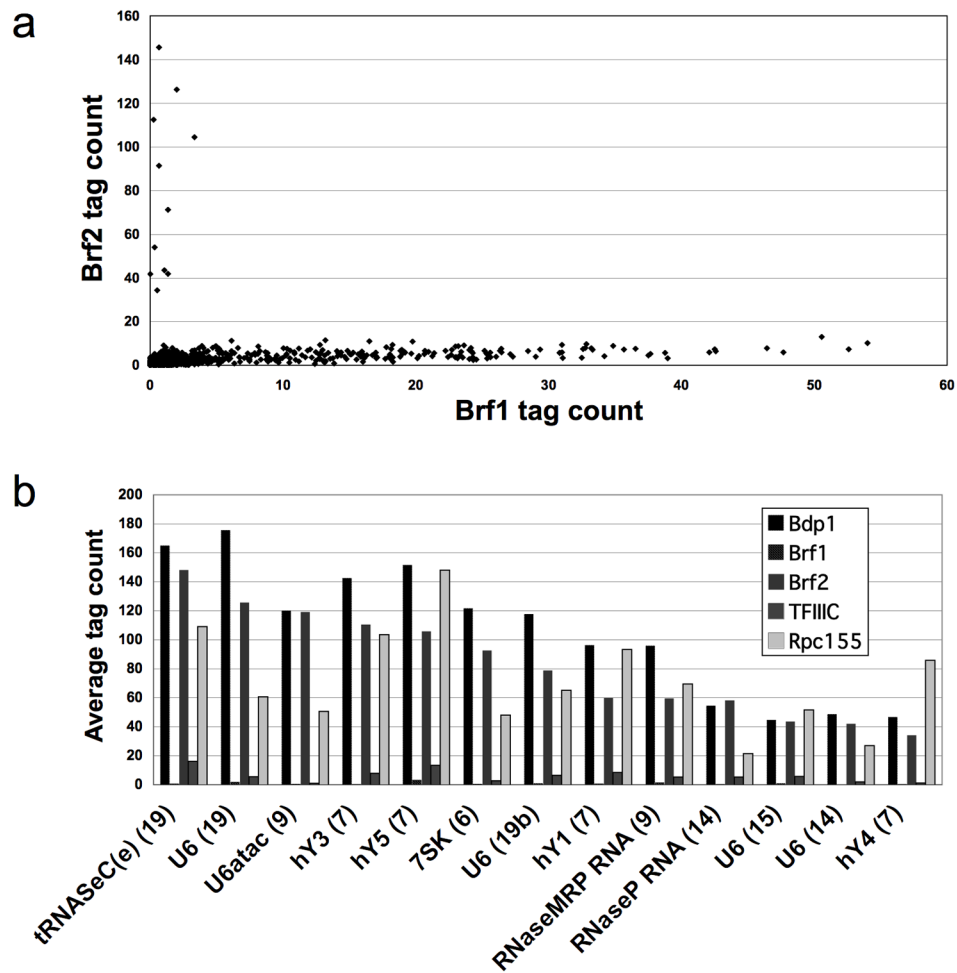


Figure 3. Distinctions between TFIIIB isoforms. **(a)** Pairwise comparison of Brf1 and Brf2 occupancy at Rpc155 peaks. The average number of sequence reads for Brf1 and Brf2 are compared for the 300bp window centered at each Rpc155 summit. **(b)** Tag counts for all five proteins at Brf2 targets. Tag counts are shown for each protein within a 300bp window centered at the summit of every indicated Brf2 peak (the chromosome number of each locus is indicated in parentheses). We show only those peaks with above-background levels of Rpc155. Rpc155 tag counts were divided by 25 to allow use of a single scale for viewing all proteins.

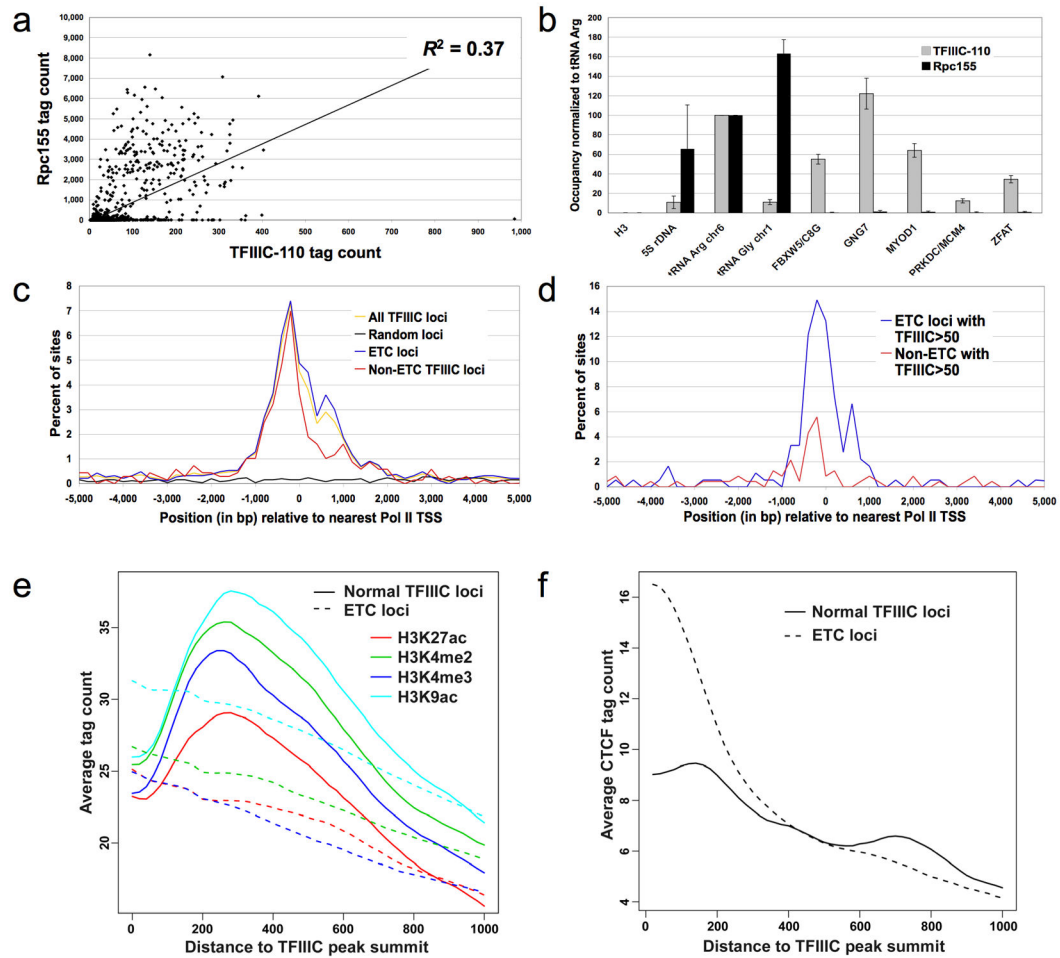


Figure 4. Relationship of TFIIIC occupancy to Pol III occupancy and identification of *ETC* loci. **(a)** Correlation of Rpc155 and TFIIIC-110 occupancy at TFIIIC-110 peaks. Comparison of the number of Rpc155 and TFIIIC-110 sequence reads within the 300 bp window centered on each TFIIIC-110 peak summit. **(b)** Pol III and TFIIIC-100 association (\pm s.d.) at typical (left) and *ETC* (right) loci by quantitative PCR in real-time. **(c)** *ETC* loci are near the TSS of Pol II genes. Frequency distribution of TFIIIC-110 peak summits with respect to the nearest Pol II TSS. **(d)** Frequency distribution of *ETC* and non-*ETC* TFIIIC-110 peaks with the highest TFIIIC levels (average tag counts >50 in the 300bp-window around the peak summit) with respect to Pol II TSS. **(e)** Positions of histone modifications relative to TFIIIC-110 peak summits at non-*ETC* (solid lines) or *ETC* (dashed lines). **(f)** CTCF tag count as a function of distance from TFIIIC-110 peak summits for non-*ETC* (solid lines) or *ETC* (dashed lines) loci.

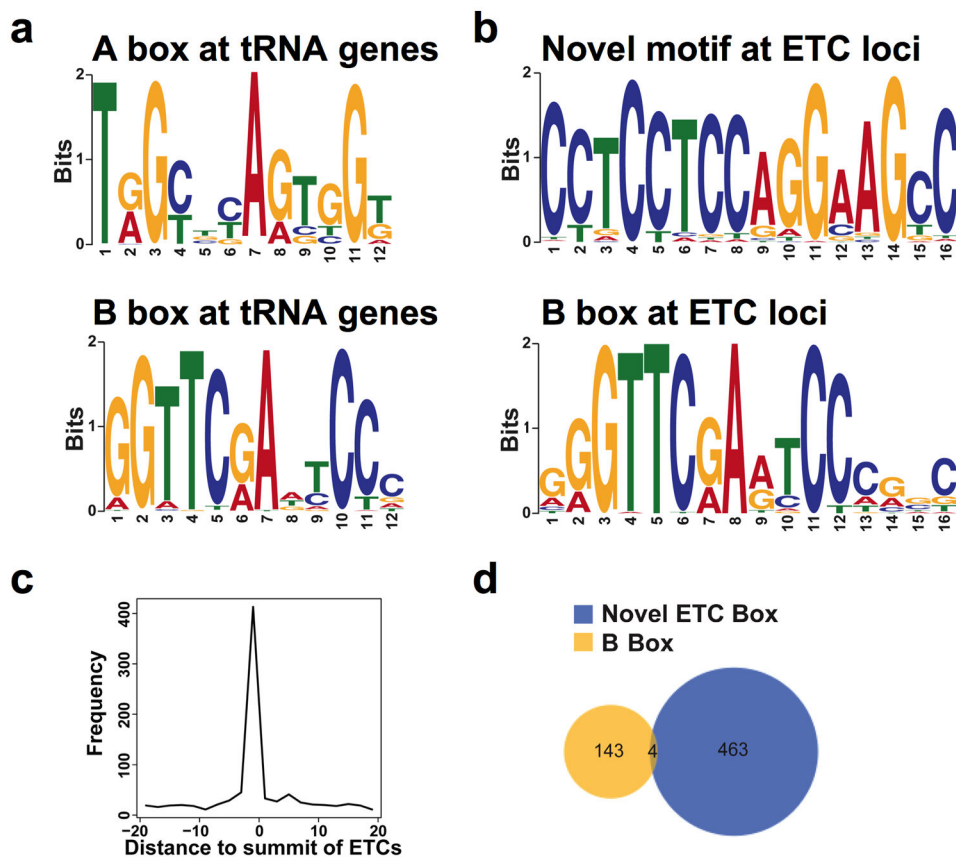


Figure 5. *ETC* motifs. (a) Known A and B box motifs found by *de novo* motif searching of tRNA genes. (b) Motifs found by *de novo* searching of *ETC* loci are a novel motif (top) and the B box motif (bottom). (c) Frequency plot of the novel *ETC* motif position as a function of distance from the TFIIC-110 peak summit. (d) Venn diagram illustrating very little overlap between TFIIC-110 peaks with the B box motif and those sites with the *ETC* motif.

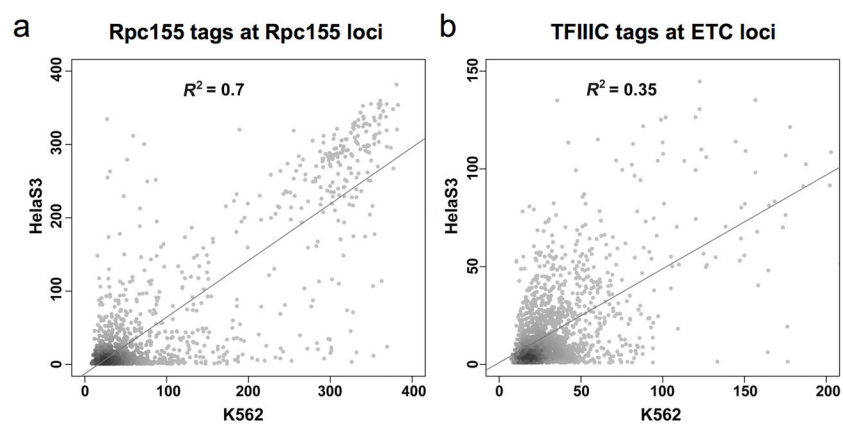


Figure 6. Cell-specificity of *ETC* loci. **(a)** Correlation of Rpc155 tag counts in K562 and HeLa cells at Rpc155-occupied loci. **(b)** Correlation of TFIIC-110 tag counts in K562 and HeLa cells at *ETC* loci.