Figures and figure supplements

Long non-coding RNA produced by RNA polymerase V determines boundaries of heterochromatin

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Figure 1. Genome-wide identification of RNA produced by Pol V (Pol V transcripts). (A) A genomic region giving rise to Pol V transcripts. The screenshot shows sequencing reads from both repeats of Pol V RIP-seq as well as Pol V ChIP-seq (Wierzbicki et al., 2012), DNA methylation (Stroud et al., 2013), and annotations of genes and Pol V transcripts. (B) Pol V RIP signal is largely limited to identified Pol V transcripts. All annotated Pol V transcripts were scaled to uniform lengths and average Pol V RIP signal from both biological repeats combined (Col-0/nrpe1, [RPM]) was plotted. The heatmap below shows Pol V RIP signal on individual transcripts sorted by length. The p value was calculated using the permutation test by comparing 100 nt long regions starting 200 nt upstream and 50 nt downstream of 5’ ends of the annotated transcripts. (C) Pol V binding to chromatin is enriched on Pol V transcripts. Profile of average Pol V ChIP-seq signal (Col-0/nrpe1 [RPM]) on scaled Pol V transcripts ± 300 bp. The p value was calculated using the permutation test by comparing 100 nt long regions starting 200 nt upstream and 50 nt downstream of 5’ ends of the annotated transcripts. (D) Pol V RIP-seq signal is enriched on regions where Pol V binds chromatin. Profiles of average Pol V ChIP-seq signal (Col-0/nrpe1) and Pol V RIP signal (Col-0/nrpe1) on Pol V ChIP-seq peaks (Wierzbicki et al., 2012) aligned with their summits ± 600 bp (10 bp resolution). (E-G) Loci generating Pol V transcripts are bound by Pol V and are targets of RdDM. Boxplots show regions producing Pol V transcripts but not overlapping ChIP-seq peaks and vice versa (RIP only and ChIP only, respectively) and on Pol V transcript regions overlapping ChIP peaks (overlap). Significance has been tested using the Wilcoxon test. (E) Pol V ChIP-seq (Col-0/nrpe1 [RPM]), (F) Pol V RIP-seq (Col-0/nrpe1 [RPM]) and (G) CHH DNA methylation (Col-0 - nrpe1).
Figure 1 continued

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Figure 1—figure supplement 1. Genome-wide identification of RNA produced by Pol V (Pol V transcripts). (A) Genomic regions giving rise to Pol V transcripts. The screenshots show sequencing reads from both repeats of Pol V RIP-seq as well as Pol V ChIP-seq (Wierzbicki et al., 2012). DNA methylation and Pol V ChIP-seq comparisons are made with the Col-0 strain. (B) Log2 Pol V RIP (Col-0 - nrpe1). [RPM], first repeat. (C) Log2 Pol V RIP (Col-0 - nrpe1). [RPM], first repeat. (D) Log2 Pol V RIP (Col-0 - nrpe1). [RPM], second repeat. (E) Log2 Pol V RIP (Col-0 - nrpe1). [RPM], first repeat. (F) Log2 Pol V RIP (Col-0 - nrpe1). [RPM], second repeat.
methylation (Stroud et al., 2013), annotations of genes (TAIR10), and annotation of Pol V transcripts obtained in this study. (B) Correlation between both biological repeats of RIP-seq. Scatterplot shows total Pol V RIP signal obtained from the first and the second repeat on annotated Pol V transcripts. Colors correspond to p values obtained using the negative binomial test included in the transcript calling protocol. (C) Pol V RIP signal is largely limited to identified Pol V transcripts – first biological repeat only. All annotated Pol V transcripts were scaled to uniform lengths and average Pol V RIP signal from the first biological repeat (Col-0/nrpe1, [RPM]) was plotted. The heatmap below shows Pol V RIP signal on individual transcripts sorted by length. Gray box on the x-axis indicates the position of the Pol V transcripts. In the heatmap every row represents an individual Pol V transcript sorted by size. The p value was calculated using the permutation test by comparing 100 nt long regions starting 200 nt upstream and 50 nt downstream of 5’ ends of the annotated transcripts. (D) Pol V RIP signal is largely limited to identified Pol V transcripts – second biological repeat only. All annotated Pol V transcripts were scaled to uniform lengths and average Pol V RIP signal from the second biological repeat (Col-0/nrpe1, [RPM]) was plotted. The heatmap below shows Pol V RIP signal on individual transcripts sorted by length. Gray box on the x-axis indicates the position of the Pol V transcripts. In the heatmap every row represents an individual Pol V transcript sorted by size. (E, F) Transcripts associated with Pol V are Pol V-dependent. RT-qPCR for specific Pol V transcripts in Col-0 and nrpe1. Average signal levels relative to wild type and standard deviations from three biological replicates are shown.

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Figure 2. Pol V regulatory elements. (A) Pol V transcripts are produced from both pericentromeric regions and chromosome arms. The number of mRNAs, transposons (TAIR10) or Pol V transcripts was plotted on chromosome 1 in 500 kb windows. (B) Pol V transcripts are significantly enriched on promoters, intergenic sequences, and transposons of all families except LTR transposons. Plots show ratios of features overlapping Pol V transcripts to those overlapping randomized genomic regions. Promoters are defined as regions 1 kb upstream of the transcription start site of genes. Stars denote significant differences based on permutations (p<0.001). (C) CG methylation is not sufficient to mediate Pol V transcription. Genes annotated in TAIR10 were split into four categories based on the presence of CHH methylation (<2%) and CG methylation (>10%). Enrichment of annotated Pol V transcripts on those categories of genes was calculated by comparing the actual overlap with overlaps of random genomic loci. Stars denote p<0.004. (D) MET1-dependent CG methylation is enriched within Pol V-transcribed regions. Average CG methylation levels (Stroud et al., 2013) within differentially methylated regions (DMRs) were plotted on scaled Pol V transcripts. The p value was calculated using the permutation test by comparing 100 nt long regions starting 200 nt upstream and 50 nt downstream of 5’ ends of the annotated transcripts. (E) A repressive histone modification is enriched on Pol V transcribed regions. Profiles of average enrichment of the modified histone (H3K9me2 and H3K4me2) over histone H3 were plotted on scaled Pol V transcripts ± 300 bp. Enrichment of H3K9me2 and depletion of H3K4me2 were statistically significant (p<0.0066 and p<0.0001, respectively, permutation test). (F) Pol V transcribes bidirectionally. Profiles of averaged Pol V RIP-seq signal (Col-0/nrpe1) in forward (grey, Figure 1B) or reverse orientation (red) on scaled Pol V transcripts ± 300 bp. Forward strand refers to annotated transcripts, reverse strand Figure 2 continued on next page.