Pervasive Transcription of Mitochondrial, Plastid, and Nucleomorph Genomes across Diverse Plastid-Bearing Species

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Abstract

Organelle genomes exhibit remarkable diversity in content, structure, and size, and in their modes of gene expression, which are governed by both organelle- and nuclear-encoded machinery. Next generation sequencing (NGS) has generated unprecedented amounts of genomic and transcriptomic data, which can be used to investigate organelle genome transcription. However, most of the available eukaryotic RNA-sequencing (RNA-seq) data are used to study nuclear transcription only, even though large numbers of organelle-derived reads can typically be mined from these experiments. Here, we use publicly available RNA-seq data to assess organelle genome transcription in 59 diverse plastid-bearing species. Our RNA mapping analyses unraveled pervasive (full or near-full) transcription of mitochondrial, plastid, and nucleomorph genomes. In all cases, 85% or more of the organelle genome was recovered from the RNA data, including noncoding (intergenic and intronic) regions. These results reinforce the idea that organelles transcribe all or nearly all of their genomic material and are dependent on post-transcriptional processing of polycistronic transcripts. We explore the possibility that transcribed intergenic regions are producing functional noncoding RNAs, and that organelle genome noncoding content might provide raw material for generating regulatory RNAs.

Key words: mitochondrial transcription, noncoding RNA, organelle gene expression, pervasive transcription, plastid transcription.

Introduction

Organelle genomes can be extreme at both the DNA and RNA levels (Smith and Keeling 2015; Smith and Keeling 2016). Gene fragmentation (Barbrook et al. 2010), gene and chromosome number variation (Shao et al. 2012; Janouškové et al. 2013), diverse genome topology (e.g., circular or linear with telomeres; Bendich 2007), and genome size range (Sloan et al. 2012) are some of the many examples of organelle genomic diversity. Similarly, the expression of organelle genomes can be unconventional, including noncanonical genetic codes (Burger et al. 2003), substitutional or insertion/deletion RNA-editing (Castandet and Araya 2011), transsplicing followed by polyadenylation (Vícek et al. 2011), and even translational bypassing (Masuda et al. 2010; Lang et al. 2014). In many instances, unraveling these complicated genomic and transcriptional architectures took years of laborious investigation, using a wide range of molecular biology techniques (Sanitá Lima et al. 2016).

More recently, next generation sequencing (NGS) has allowed researchers to take a genome-wide approach to investigating organelle genomes and transcriptomes (Ruwé et al. 2013). For instance, high-throughput RNA sequencing (RNA-seq) of isolated organelles helped uncover pervasive transcription in the human mitochondrial genome and barley plastid genome (Mercer et al. 2011; Zhelyazkova et al. 2012). Given the popularity of NGS, organelle transcription can now easily be explored using publicly available RNA-seq data from whole-cell experiments (Smith 2013). Indeed, such an approach revealed full transcription of plastid DNAs (ptDNAs) from various land plants (Shi et al. 2016) and in the mitochondrial DNAs (mtDNAs) of Polytomella green algae (Tian and Smith 2016).
Most of the researchers that generate whole-cell eukaryotic RNA-seq data are not necessarily interested in organelle transcription, and many treat the organelle-derived reads as contamination, filtering them out before downstream analyses. Consequently, public databases, such as the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), are increasingly becoming an untapped source for organelle transcriptomic data from eukaryotic RNA-seq experiments, regardless of the NGS sequencing protocol that was used (Smith and Sanitá Lima 2016).

RNA-seq data alone are rarely enough to uncover the full complexity of organelle gene expression, but they are a fast, efficient, and cost-effective first approach to studying transcription (Dietrich et al. 2015). Although pervasive transcription has been extensively demonstrated in nuclear and bacterial systems (Berretta and Morillon 2009; Wade and Grainger 2014), it is not yet known how common this process is among organelle genomes. Most of the reports of genome-wide transcription in organelles come solely from model species (Hutto et al. 2012; Ro et al. 2013; Ross et al. 2016), suggesting that this strategy is the norm, rather than the exception, in mitochondria and plastids, and perhaps inherited from their bacterial progenitors (Shi et al. 2016). So, is pervasive transcription a common theme among mtDNAs and ptDNAs across the eukaryotic domain? And do compact versus bloated organelle genomes differ in their transcriptional patterns?

Here, by taking advantage of publicly available eukaryotic RNA-seq data, we investigate the transcriptional architecture of diverse plastid-bearing species, and show that pervasive transcription is a widespread phenomenon across the eukaryotic domain, including in very large organelle genomes with high noncoding contents. We speculate about the potential function roles (if any) of organelle noncoding RNAs (ncRNAs), particularly with respect to land plants and mixotrophs. If anything, these data highlight the utility of freely accessible RNA-seq data for organelle gene expression studies.

Materials and Methods

Using the NCBI Taxonomy Browser (https://www.ncbi.nlm.nih.gov/taxonomy, last accessed July 10, 2017), we identified 59 plastid-bearing species for which complete mitochondrial, plastid, and/or nucleomorph genome sequences (>100 kb) and ample RNA-seq data sets were available. We limited our search to species with organelle genomes that were >100 kb or greater. Previously, we explored the prevalence of pervasive transcription in small and compact organelle genomes (<105 kb; Sanitá Lima and Smith 2017), and here we wanted to see if the same trends held for larger organelle DNAs with long intergenic regions.

The 59 species we identified include land plants and other members of the Archaeplastida as well as various species with “complex” plastids, such as cryptophytes and stramenopiles (supplementary table S1, Supplementary Material online). The organelle genomic architectures of these species span the gamut of size (~104–980 kb), coding content (~0.6–82%), structure (circular vs. linear), and chromosome number (intact vs. fragmented). The RNA-Seq data were downloaded from the NCBI SRA (Kodama et al. 2012), and the genome sequences from GenBank. See supplementary table S1, Supplementary Material online for detailed information on the RNA-seq and organelle genome data we collected, including accession numbers, read counts, sequencing technologies, organelle genome features (e.g., GC content, genome topology, and percent protein-coding), and the strains used for genome and transcriptome sequencing.

We ensured that the RNA-seq and corresponding organelle genome data came from the same species, but sometimes they came from different strains of the same species (supplementary table S1, Supplementary Material online). Also, the RNA-seq experiments we sourced were often generated using very different protocols and experimental conditions (supplementary table S1, Supplementary Material online). Nevertheless, these caveats did not hinder the mapping analyses (see below).

Mapping analyses were performed using Geneious v9.1.6 (Biomatters Ltd., Auckland, NZ; Kearse et al. 2012). Briefly, raw whole-cell RNA-seq reads were mapped to the corresponding organelle genomes with Bowtie 2 (Langmead and Salzberg 2012) using the default settings, the highest sensitivity option, and a min/max insert size of 50 nt/750 nt. We allowed each read to be mapped up to two locations to account for repeated regions, which are common in organelle genomes (Smith and Keeling 2015). The mapping histograms were extracted from Geneious.

Results

Pervasive Transcription Is Widespread across Organelle and Nucleomorph Genomes

For each of the organelle genomes studied here, RNA-seq reads covered 85% or more of the reference sequence (RefSeq), regardless of the genome size, noncoding content, or taxonomic grouping (fig. 1, and supplementary table S1 and fig. S1, Supplementary Material online). In 24 cases, >99% of the organelle DNA sequence was present at the RNA level. In other words, all of the genomes exhibited pervasive, genome-wide transcription. The mean RNA-seq read coverage was consistently high across the different genomes, varying from ~30 to >2,300,000 reads/nt.

Together, these data indicate that noncoding regions from disparate organelle genomes are broadly transcribed, which can be clearly deduced from the RNA-seq mapping histograms (supplementary fig. S1, Supplementary Material online). This was true for relatively compact genomes, such as the ptDNA of the stramenopile alga Nannochloropsis oceanica (82% coding; RefSeq coverage 94%) as well as for the
FIG. 1.—Occurrence of pervasive transcription in mitochondrial, plastid and nucleomorph genomes across plastid-bearing species. Unscaled phylogenetic relationships were adapted accordingly from different studies: overall angiosperm phylogeny (Stevens 2001), Milioidioid sensu late clade phylogeny (Wojciechowsk 2006), major eukaryotic groups phylogeny (Burki 2014), ferns phylogeny (Plackett et al. 2015) and Cucurbitaceae phylogeny (Renner and Schaefer 2016) mt, mitochondrion; pt, plastid; cy, cyanelle; nm, nucleomorph; RefSeq %, percentage of the reference organelle genome covered by one or
highly bloated organelle genomes (fig. 1 and supplementary table S1 and fig. S1, Supplementary Material online). For instance, RNA-seq coverage exceeded 90% for the very large mitochondrial genomes of the land plants *Salvia miltiorrhiza* (~499 kb, ~9.5% coding), *Capsicum annuum* (~507 kb, ~12% coding), *Rhazya stricta* (~548 kb, ~8% coding), *Asclepias syriaca* (~682 kb, ~5% coding), *Phoenix dactylifera* (~715 kb, ~5% coding), and *Cucurbita pepo* (~982 kb, ~15% coding; fig. 2). This implies that hundreds of thousands of nucleotides of ncRNAs are being generated in these mitochondria, and within distinct groups of angiosperm (e.g., asterids, commelinids, and rosids).

In fact, pervasive transcription of mitochondrial and plastid genomes appears to be the norm rather than the exception across plastid-bearing species as a whole. We found that it was common throughout the Archaeoplastida, including in land plants, green algae, red algae, and glaucophytes, as well as in species with euakaryote-eukaryote derived plastids. Complete or nearly complete transcription is also found in organisms coming from very different habitats and ecosystems, such as deserts (e.g., *Welwitschia mirabilis*), irriated cultures (e.g., *Zea mays* and *Glycine max*), freshwater (e.g., *Tetraselmis obliquus*), and seawater (e.g., *Pyropia spp.*).

Among the most impressive examples of pervasive organelle transcription comes from the mtDNA of the dinoflagellate alga *Symbiodinium minutum*, a coral symbiont (Coffroth and Santos 2005). This ~326 kb genome is made up of >99% noncoding DNA, all of which appears to be transcriptionally active (fig. 1 and supplementary table S1 and fig. S1, Supplementary Material online). This result is consistent with a previous report of full mitochondrial transcription of the *S. minutum* mitochondrial genome using a different data set (Shoguchi et al. 2015). We also observed full transcription in the nucleomorph genomes of *Cryptomonas paramecium* and *Hemiselmis andersenii* (fig. 3).

**Discussion**

Our RNA mapping analyses provide various insights into organelle transcription and how it can be investigated using publicly available RNA-seq data. First, the size of the RNA-seq data sets we employed did not always positively correlate with the overall organelle genome read coverage (supplementary table S1, Supplementary Material online). This was to be expected given that the RNA-seq data we used came from different experiments and laboratory groups and were produced under varying conditions and sequencing protocols. Poly-A selection, for example, can lead to an enrichment in highly AT-rich organelle transcripts, and in some lineages, including land plants, organelle polyadenylation is a target for transcript degradation (Small et al. 2013). But we quickly overcame any issues associated with biased or underrepresented organelle reads by combining multiple RNA-seq data sets from different experiments (supplementary table S1, Supplementary Material Online).

We also found differences in the RNA-seq coverage statistics for plastid and mitochondrial genomes. For the species which we had complete sequence data for both the mitochondrial and plastid genomes, the latter tended to have higher overall and mean coverage rates than the former. This could be connected to transcript abundance or genome copy number of plastids versus mitochondria, or perhaps the half-life of mitochondrial transcripts is shorter than that of plastid RNAs, or merely that mitochondria are responding to the experimental treatments differently than plastids.

In some instances, organelle genome intergenic regions were not completely represented in the RNA-seq data (i.e., RefSeq coverage <100%). This is possibly a consequence of post-transcriptional processing resulting in the cleavage of those regions, thus, preventing them from being captured in the transcriptomic sequencing experiment. But even when considering these few missing regions, there is no denying that organelle genomes typically go full transcription no matter their structure, size, content, or taxonomic grouping.

Many of the genomes we analyzed undergo minor to moderate amounts of substitutional RNA editing (Shoguchi et al. 2015; Shi et al. 2016). We did not set out to specifically study post-transcriptional editing, but we were able to easily identify edited sites from our mapping analyses, reinforcing the utility of freely available RNA-seq for quantifying and categorizing RNA editing in organelle systems (Smith 2013; Moreira et al. 2016; Shi et al. 2016). Micro-RNA (miRNA) analyses were also beyond the scope of our work, but nevertheless we covered 4.5% of the *Citrullus lanatus* (watermelon) mitochondrial genome using only a few micro-RNA NGS data sets (data not shown). Telomeric RNA can be studied using RNA-seq: we found widespread telomeric transcription of the nucleomorph genomes from *C. paramecium* and *H. andersenii*, which is in line with previous work on the mitochondrial telomeres of *Polytomella* spp. (Tian and Smith 2016) and apicomplexan parasites (Raabe et al. 2010). The significance of organelle telomeric transcription is not known, but in the nuclei of humans, mice, yeast, and zebrafish, telomeres can be transcribed into regulatory long ncRNAs called TERRA (telomeric repeat-containing RNA; Arora et al. 2012; Maicher et al. 2012; Cusanelli and Chartrand 2015).
The utility of RNA-seq for scrutinizing organelle gene expression has its limitations and drawbacks. For example, nuclear mitochondrial-like and nuclear plastid-like DNA (NUMTs and NUPTs)—and even mitochondrial plastid-like DNA—

**Fig. 2.**—Full transcription of bloated mitochondrial genomes in land plants. Mapping histograms show coverage depth (transcripts mapped per nucleotide) on a log scale. Organelle genome annotations are from genome assemblies deposited in GenBank (accession numbers provided in supplementary table S1, Supplementary Material online). Mapping contigs are not to scale and direction of transcription is given by the arrows of the annotated genes. Mapping histograms were extracted from Geneious v9.1.6 (Kearse et al. 2012).

**Fig. 3.**—Full transcription of nucleomorph genomes in cryptophytes. *Cryptomonas paramecium* and *Hemiselmis andersenii* had full transcription for each one of the three chromosomes in their nucleomorph genomes, including telomeric regions. Mapping histograms follow the same structure as in figure 2; mapping contigs are not to scale.

The utility of RNA-seq for scrutinizing organelle gene expression has its limitations and drawbacks. For example, nuclear mitochondrial-like and nuclear plastid-like DNA (NUMTs and NUPTs)—and even mitochondrial plastid-like DNA
teria, including alphaproteobacteria and cyanobacteria (Landt et al. 2008; Georg et al. 2009; Schlueter et al. 2010; Mitschke et al. 2011a, 2011b; Voigt et al. 2014). Therefore, its widespread occurrence in organelles brings more and more examples of complete organelle genome transcription from disparate groups throughout the eukaryotic tree of life. However, the functional relevance of this is poorly understood (Vendramin et al. 2017). The transport of RNA is even more complicated in the case of complex plastids (Keeling 2013), which could likely be generated and regulated in part by ncRNAs. Similar arguments can be made for the evolution of mixotrophic algae, which can switch between heterotrophy and photoautotrophy (Jassey et al. 2015). Although speculative, the mechanisms for trophic mode determination could be partly controlled by organelle (or nuclear) ncRNAs generated via pervasive transcription. It would be interesting to explore the hypothesis that organelle genome size variation (together with organelle number) played a role in the evolution of mixotrophy. After all, noncoding sequences can be used as the raw material for generating new regulatory pathways (Libri 2015).

Although not the first account of pervasive organelle transcription, this is the first report to show such widespread occurrence of this phenomenon. Most of the data used in our work came from whole-cell RNA-seq experiments in which the organelle reads were ignored. That we could use these data to assemble complete or near-complete organelle transcriptomes highlights the value of publicly available RNA-seq experiments (and the SRA) for organelle research. This work...
also emphasizes the ease at which one can assemble a complete organelle genome from RNA-seq data alone. A quick scan through the SRA reveals many species for which there are whole-cell RNA-seq data but no or minimal organelle DNA sequence data (Smith and Sanit a Lima 2016). Some of these species are poorly studied marine protists of great ecological importance, which had their transcriptomes sequenced as part of the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP; Keeling et al. 2014). As a proof of concept, 14 land plant plastid genomes were recently de novo assembled from transcriptomic data coming from SRA (Shi et al 2016). Clearly, publicly available whole-cell RNA-seq data are a goldmine for organelle genomics and transcriptomics (Smith 2013). We just need to start digging.

Supplementary Material
Supplementary data are available at Genome Biology and Evolution online.

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