

## ***C. elegans* noncoding RNA genes**

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The *C. elegans* genome contains approximately 1300 genes that are known to produce functional noncoding RNA (ncRNA) transcripts, as opposed to mRNAs that encode proteins. These known ncRNA genes include about 590 transfer RNA (tRNA) genes, 275 ribosomal RNA (rRNA) genes, 140 trans-spliced leader RNA genes, 120 microRNA (miRNA) genes, 70 spliceosomal RNA genes, and 30 snoRNA genes.

Based on what is known about ncRNA-directed functions in other animals, there are additional ncRNA genes performing known biochemical functions that have not yet been identified in the worm genome. These include the telomerase RNA and on the order of 100-200 small nucleolar RNA (snoRNA) genes that direct site-specific 2'-O-ribose methylations and pseudouridylations of ribosomal RNAs and other target RNAs.

It also seems likely that novel ncRNA genes remain to be discovered. The belated realization that the *lin-4* and *let-7* regulatory RNA genes are not just worm-specific anecdotes, but instead are members of a huge gene family of microRNAs with important roles in posttranscriptional gene regulation in many eukaryotes (Lee et al., 1993; PMID 8252621; Lim et al., 2003; PMID 12672692; Pasquinelli et al., 2000; PMID 11081512; Reinhart et al., 2000; PMID 10706289) was a spectacular demonstration that important genes (indeed, whole gene families) can easily escape standard computational and experimental gene discovery methods. There is a tantalizing possibility that the miRNAs foreshadow the discovery of even more RNA-directed functions.

Here we describe what is currently known about the ncRNA genes of *C. elegans*, from the perspective of the annotation of the finished genome sequence (Consortium, 1998; PMID 9851916). Based on the literature, Genbank, and on computational searches for homologs of known RNAs and members of known RNA gene families (Benson et al., 2004; PMID 14681350; Griffiths-Jones, 2004; PMID 14681370; Griffiths-Jones et al., 2003; PMID 12520045; Harris et al., 2004; PMID 14681445; Lowe and Eddy, 1997; PMID 9023104), we have collated a stable, curated reference set of *C. elegans* noncoding RNA genes, and their chromosomal coordinates relative to the WS130 version of the genome sequence assembly in [Table 1](#). We have made these data available as annotation tracks for WormBase, and downloadable as HTML tables, GFF coordinate files, or FASTA sequence files. We describe how the reference set has been produced, and summarize what it contains.

### ***Ribosomal RNAs***

The 18S, 5.8S, and 26S subunits of rRNA are transcribed by RNA polymerase I from a 7.2 kb rDNA unit that is tandemly repeated ~55 times at the end of chromosome I (Consortium, 1998; PMID 9851916; Ellis et al., 1986; PMID; Sulston and Brenner, 1974; PMID 4858229). The 5S rRNAs are transcribed separately by RNA polymerase III from ~110 copies of a ~1 kb tandem repeat unit on chromosome V (Nelson and Honda, 1985; PMID 4065574; Sulston and Brenner,

1974; PMID 4858229). (The 5S rRNA repeat unit also includes the gene for the SL1 trans-spliced leader; see below.) The rRNA genes are systematically underrepresented in the current genome sequence assembly, because tandem arrays are problematic for physical mapping and sequencing. According to WUBLAST searches using the published sequences of the 7.2 kb and 1 kb rDNA repeat units as queries, one copy of the 18S/5.8S/26S rRNA repeat unit is represented in the chromosome I sequence assembly, and fifteen copies of the 5S rRNA gene are included in the chromosome V sequence. Additionally, the mitochondrial DNA contains one 18S rRNA gene and one 23S rRNA gene.

## ***Transfer RNAs***

We have annotated genes for 569 nuclear tRNAs, 22 mitochondrial tRNAs, and 1072 probable tRNA pseudogenes. The mitochondrial tRNAs were curated from the literature (Okimoto et al., 1992; PMID 1551572; Wolstenholme et al., 1987; PMID 3469671). Nuclear tRNAs can be reliably identified by computational methods. We used the programs tRNAscan-SE (Lowe and Eddy, 1997; PMID 9023104) and ARAGORN (Laslett and Canback, 2004; PMID 14704338) to identify a combined candidate list of 612 putative tRNA genes and 214 candidate tRNA pseudogenes. These candidate tRNA genes were manually curated to remove an additional 40 putative pseudogenes and 3 false positives, leaving the final set of 569 annotated genes. This gene set is essentially in agreement with the independent analysis of Marck and Grosjean, who identified 529 putative tRNA genes (Marck and Grosjean, 2002; PMID 12403461). Differences appear to be due to variation in what is called a putative true gene versus a putative pseudogene, and differences in the version of the genome assembly used.

As is the case in many eukaryotes, tRNA pseudogenes are numerous in *C. elegans*. Current tRNA scanning programs only detect pseudogenes that are closely related to true tRNAs. Using WUBLAST, we identified an additional 818 sequences with significant similarity to one or more of the 569 tRNA genes and/or 254 tRNA pseudogenes, and added them to annotate a total of 1072 putative pseudogenes. Many of these overlay four previously identified repetitive sequences (Tc4, CEREP3, CELE45, and NDNAX3\_CE) defined by RepBase and RepeatMasker searches.

## ***Spliced leader RNAs***

Approximately 70% of *C. elegans* mRNAs are covalently modified at their 5' end by the addition of 22-nt trans-spliced leader RNA sequences (Blumenthal and Gleason, 2003; PMID 12560808; Ross et al., 1995; PMID 7665629; Zorio et al., 1994; PMID 7969472). Trans-spliced leaders are donated by independently transcribed ~100-110 nt SL RNAs, which come in two forms, SL1 RNA and SL2 RNA. The most abundant form, SL1, is predominantly trans-spliced to the 5' end of pre-mRNAs, including the first cistron in polycistronic (operon) pre-mRNAs; the rarer form, SL2, is generally trans-spliced to downstream cistrons in polycistronic operons (Blumenthal and Gleason, 2003; PMID 12560808). The genes for SL1 RNA are part of the same tandem repeat unit that encodes 5S rRNA, occurring in ~110 copies on chromosome V (Krause and Hirsh, 1987; PMID 3581169; Nelson and Honda, 1985; PMID 4065574). Ten SL1 RNA genes are represented in the

current genome sequence assembly. The genes for the ~110 nt SL2 RNAs are dispersed, and show more sequence variation than SL1 RNAs (indeed, some have been named SL3 RNA, SL4 RNA, etc.; we follow the WormBase convention of annotating all as SL2 variants) (Huang and Hirsh, 1989; PMID 2813415; Ross et al., 1995; PMID 7665629; Zorio et al., 1994; PMID 7969472). 20 SL2 RNA gene variants are found in the genome sequence, roughly in agreement with the copy number of ~30 predicted from genomic Southern blots (Ross et al., 1995; PMID 7665629; Zorio et al., 1994; PMID 7969472). SL RNAs are thought to be transcribed by pol II (Krause and Hirsh, 1987; PMID 3581169).

## ***Small nucleolar RNAs (snoRNAs)***

In Eukarya and Archaea, two classes of snoRNAs direct site-specific base modifications of ribosomal RNA and other ncRNAs. C/D box snoRNAs direct 2'-O-ribose methylations, and H/ACA snoRNAs direct pseudouridylations. The modifications are catalyzed by snoRNA-associated proteins (fibrillarin in the case of 2'-O-ribose methylation, Cbf5/dyskerin in the case of pseudouridylation); the snoRNAs function to guide a snoRNP complex to a modification site by complementary base pairing between the snoRNA and the target RNA. One snoRNA usually targets one or two modifications, and any given modification site can be targeted by more than one redundant snoRNA. The best-studied targets for snoRNA-directed base modification, ribosomal RNAs, have about 40-100 2'-O-ribose methylations and a comparable number of pseudouridylations in eukaryotes such as yeast and human. We expect a similar number of modifications in *C. elegans* rRNAs, and therefore expect to find on the order of 100-200 rRNA modification guide snoRNAs in the genome (and probably additional snoRNAs that direct modifications of other ncRNAs).

However, snoRNAs are difficult to identify from sequence analysis alone, and we have only a partial map of *C. elegans* rRNA modifications (Higa et al., 2002; PMID 12372436), so the catalog of *C. elegans* snoRNA genes is currently incomplete. We can annotate 19 C/D and 6 H/ACA snoRNA genes based on Genbank and the current literature (Higa et al., 2002; PMID 12372436; Wachi et al., 2004; PMID 15194189).

In vertebrates, almost all known C/D and H/ACA snoRNAs are processed out of introns of host genes, whereas in yeast and plants, snoRNAs are usually independently transcribed (Tycowski et al., 2004; PMID 15556860). *C. elegans* likely uses a mix of the two expression strategies. Many of the 2'-O-ribose methylation-guide C/D box snoRNAs are preceded by conserved proximal sequence elements (PSEs) typical of snRNA promoters (Thomas et al., 1990; PMID 2339054). The H/ACA snoRNAs identified by Wachi et al. were found in the introns of protein-coding genes (Wachi et al., 2004; PMID 15194189).

A small number of additional snoRNAs do not direct nucleotide modifications. An important one is the U3 snoRNA, which is involved in endonucleolytic processing of the 18S/5.8S/26S rRNA precursor. Six U3 snoRNA genes have been identified by sequence similarity searches (TA Jones, SR Eddy, unpublished).

## ***microRNAs (miRNAs)***

We annotated both the putative *Drosha*-produced pre-miRNA (~60nt) and the *Dicer*-produced miRNA (~21nt) forms of each of 117 microRNAs in the Rfam miRNA Registry database (Griffiths-Jones, 2004; PMID 14681370). We also annotated 37 of the ~21-nt “tiny noncoding RNA” (tncRNA) sequences identified by Ambros et al., which appear to be produced in a *Dicer*-dependent fashion and thus are related to the miRNA/siRNA pathways (Ambros et al., 2003; PMID 12747828). See section XXX for a thorough description of *C. elegans* microRNA biology.

We have not annotated the ~700 endogenous small interfering RNAs (siRNAs) found by Ambros et al. (Ambros et al., 2003; PMID 12747828). These ~21 nt siRNAs exhibit perfect complementarity to coding regions, and thus may represent post-transcriptional gene regulation by endogenous RNAi. For a description of the RNAi phenomenon in *C. elegans*, see section XXX.

## ***Other known RNAs***

The spliceosome contains five different small nuclear RNAs (snRNAs), called U1, U2, U4, U5, and U6. Genes for the abundant spliceosomal RNAs typically occur in multiple copies in higher eukaryotes. The *C. elegans* spliceosomal RNA genes were studied by Thomas et al., who identified 21 genes (1 U1, 10 U2, 3 U4, 1 U5, and 1 U6) dispersed in the genome, and who estimated copy numbers based on genomic Southern blots of 11 U1, 12 U2, 6 U4, 9 U5, and 10 U6 snRNA genes (Thomas et al., 1990; PMID 2339054). Based on WUBLASTN searches using the Thomas et al. sequences as queries, we identify a total of 72 spliceosomal RNA loci (12 U1, 19 U2, 5 U4, 13 U5, and 23 U6), at least five of which appear to be pseudogenes.

Unlike plants and some other animals, *C. elegans* does not appear to utilize the alternative U12 spliceosome components U11, U12, U4atac, and U6atac; their homologs are not detectable in the *C. elegans* genome and no ESTs containing characteristic AT/AC splice-junctions have been found.

RNase P RNA, which catalyzes the maturation of tRNA 5' ends, is present in a single copy in the *C. elegans* genome; it was identified by computational homology searches (RJ Klein, Ph.D. thesis; SM Marquez and NR Pace, unpublished).

The ceY RNA is also present in a single genomic locus. The function of its RNP complex with Ro protein is unclear, but may be to act as a 5S rRNA quality control agent (Van Horn et al., 1995; PMID 7489501).

SRP RNA is a component of the signal recognition particle, involved in translocation of nascent proteins across the endoplasmic reticulum. Five SRP-RNA genes have been identified in the *C. elegans* genome by homology search methods (Regalia et al., 2002; PMID 12140321).

In other metazoans, the 3' ends of histone genes are processed via the interaction of a downstream stemloop with the U7 ncRNA. To date, no *C. elegans* U7 RNA homologs have been found. The 7SK RNA, a negative regulator of pol II transcription in higher mammals, also has no detectable homolog in the *C. elegans* genome.

The most important ncRNA that has yet to be identified in the *C. elegans* genome is the telomerase RNA. Similar to almost all other eukaryotes, *C. elegans* telomeres consist of 1-2 kilobases of six-nucleotide (TAAGGC) repeats (Cheung et al., 2004; PMID 15247331), which in ciliates, vertebrates, and fungi are known to be synthesized by a telomerase RNP that contains a reverse transcriptase and a telomerase RNA template, in addition to other proteins (Blackburn, 2001; PMID 11572773). Telomerase RNAs are highly diverged, and we do not expect to be able to identify a homologous RNA in *C. elegans* by BLAST searches using known telomerase RNAs as queries (and indeed, we do not). More powerful structure-based searches might be productively applied now that a satisfactory consensus secondary structure for ciliate, vertebrates, and fungi has been worked out (Lin et al., 2004; PMID 15371596). Using 3' SAGE tagging, Jones et al. detected an abundant, apparently noncoding transcript *tts-1* which was predicted to contain a plausible telomeric template sequence, leading *tts-1* to be suggested as a possible telomerase RNA (Jones et al., 2001; PMID 11483575). However, this enigmatic transcript seems unlikely to be the true telomerase RNA, as cDNA sequencing has shown that the putative telomeric template region is not part of the major *tts-1* transcript (SL Stricklin and SR Eddy, unpublished).

### ***ncRNA conservation***

Most known *C. elegans* ncRNAs are well conserved with their *C. briggsae* homologs: similarity ranges from complete identity (for 5S rRNA, U6 RNA, and SL1 RNA) to 89% identical for RNase P and 75% identical for the ceY RNA. About two-thirds of *C. elegans* miRNAs are clearly conserved in *C. briggsae*, but none of the 37 annotated *C. elegans* tncRNAs (or the immediate genomic context of these ~21mers) appear to be conserved in *C. briggsae*.

### ***Prospects for novel ncRNAs***

It must be noted that it is not currently possible to systematically identify novel noncoding RNA genes that have no homology to known genes. We do not yet have reliable “genefinding” programs for ncRNAs; current computational ncRNA genefinding approaches are suitable as screens, but not for reliable automated annotation. The best current computational methods use comparative sequence analysis to identify conserved RNA secondary structure (Coventry et al., 2004; PMID 15304649; Rivas and Eddy, 2001; PMID 11801179; Washietl et al., 2005; PMID 15665081). Genome sequencing of several related *Caenorhabditis* species is beginning to make such comparative ncRNA genefinding approaches increasingly powerful in *C. elegans*.

Systematic gene discovery by cDNA sequencing (and related transcript discovery methodologies such as SAGE and MPSS) is usually done on poly-A+, mRNA-enriched, ncRNA-depleted populations. It is possible to use these transcript discovery approaches on total RNA or ncRNA-enriched RNA populations (Huttenhofer et al., 2004; PMID 15103086), but to our knowledge such experiments have not yet been published for *C. elegans*. We also expect that whole-genome tiled expression arrays should soon make it possible to systematically catalog the entire *C. elegans* transcriptome, including both coding and noncoding transcripts (Kampa et al., 2004; PMID 14993201; Kapranov et al., 2003; PMID 15239943).

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