REPORT

Interplay between AAUAAA and the *trans*-splice site in processing of a *Caenorhabditis elegans* operon pre-mRNA

YINGMIAO LIU,^{1*} TAO HUANG,^{1,2*} MARGARET MACMORRIS,¹
and THOMAS BLUMENTHAL¹

¹Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA

ABSTRACT

About half of *Caenorhabditis elegans* genes have a 1–2 bp mismatch to the canonical AAUAAA hexamer that signals 3' end formation. One rare variant, AGUAAA, is found at the 3' end of the *mai-1* gene, the first gene in an operon also containing *gpd-2* and *gpd-3*. When we expressed this operon under heat shock control, 3' end formation dependent on the AGUAAA was very inefficient, but could be rescued by a single bp change to create a perfect AAUAAA. When AGUAAA was present, most 3' ends formed at a different site, 100 bp farther downstream, right at the *gpd-2* *trans*-splice site. Surprisingly, 3' end formation at this site did not require any observable match to the AAUAAA consensus. It is possible that 3' end formation at this site occurs by a novel mechanism—*trans*-splicing-dependent cleavage—as deletion of the *trans*-splice site prevented 3' end formation here. Changing the AGUAAA to AAUAAA also influenced the *trans*-splicing process: with AGUAAA, most of the *gpd-2* product was *trans*-spliced to SL1, rather than SL2, which is normally used at downstream operon *trans*-splice sites. However, with AAUAAA, SL2 *trans*-splicing of *gpd-2* was increased. Our results imply that (1) the AAUAAA consensus controls 3' end formation frequency in *C. elegans*; (2) the AAUAAA is important in determining SL2 *trans*-splicing events more than 100 bp downstream; and (3) in some circumstances, 3' end formation may occur by a *trans*-splicing-dependent mechanism.

Keywords: 3' end formation; nematode; RNA processing; *trans*-splicing

INTRODUCTION

In higher eukaryotes, mRNA 3' end formation is dependent on a complex of proteins including cleavage and polyadenylation specificity factor (CPSF) and cleavage stimulatory factor (CstF). CPSF binds to the AAUAAA signal just upstream of the site of 3' end formation, whereas CstF binds to a U-rich or GU-rich signal about 30 bp downstream of the 3' end formation site (reviewed in Zhao et al., 1999). In vertebrates, the upstream site is nearly always a perfect AAUAAA, but in *Caenorhabditis elegans*, a number of variants occur (Blumenthal & Steward, 1997). One rare variant, AGUAAA, used in <1% of *C. elegans* genes, is present at the 3' end of the *mai-1* gene (Lee et al., 1992).

Utilization of this rare variant is conserved in the *mai-1* gene of *Caenorhabditis briggsae*, a species that diverged from *C. elegans* about 50 million years ago, so it is likely to be functionally significant. Some of the *mai-1* mRNA is cleaved and polyadenylated immediately following the AGUAAA, but much of it extends an additional 100 nt downstream (Spieth et al., 1993), suggesting the AGUAAA could result in inefficient 3'-end formation. Both mRNAs clearly represent sites of polyadenylation, as both classes are represented in an oligo(dT)-primed cDNA library (Spieth et al., 1993).

In *C. elegans*, many genes are arranged in operons (Spieth et al., 1993; Zorio et al., 1994). These closely clustered genes are cotranscribed to produce polycistronic pre-mRNAs that are processed into mature monocistronic transcripts. This involves a combination of cleavage and polyadenylation at the 3' end of the upstream mRNA and *trans*-splicing at the 5' end of the downstream mRNA. In *trans*-splicing, a 22-nt spliced leader (SL) is transferred from the SL snRNP to the 5' ends of the mRNAs (reviewed in Blumenthal & Steward, 1997). The major spliced leader, SL1, is used when

Reprint requests to: Thomas Blumenthal, Department of Biochemistry and Molecular Genetics, Box B121, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, Colorado 80262, USA; e-mail: tom.blumenthal@uchsc.edu.

*The first two authors contributed equally to this work.

²Present address: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA

the *trans*-splice site is close to the 5' end of the pre-mRNA. A second type of SL RNP, SL2, is used exclusively for *trans*-splicing to the downstream genes in polycistronic transcripts. However, some downstream genes in operons are *trans*-spliced to both SL1 and SL2 (Blumenthal & Steward, 1997). *mai-1* is the first gene in a three-gene operon, and the gene following *mai-1*, *gpd-2*, is *trans*-spliced mostly to SL1 (Huang & Hirsh, 1989; Spieth et al., 1993).

Because genes in operons are separated by about 100–400 bp, it is possible that 3'-end formation at the upstream gene and *trans*-splicing at the 5' end of the downstream gene are functionally coupled. Indeed, elimination of 3'-end formation of an upstream gene reduced the level of SL2 *trans*-splicing to the *gpd-3* *trans*-splice site 110 nt downstream (Kuersten et al., 1997). Here we test the idea that the rare AAUAAA variant present at the 3' end of *mai-1* results in inefficient 3'-end formation and in the utilization of SL1 instead of SL2. We demonstrate that by changing the signal to a perfect AAUAAA, both 3'-end formation efficiency at the proximal site and SL2 *trans*-splicing of *gpd-2* increase. We also demonstrate that 3'-end formation at the distal site is dependent on the *trans*-splice site, but not the AGUAAA 100 bp upstream, suggesting the existence of a novel mechanism of 3'-end formation in *C. elegans* operons.

RESULTS AND DISCUSSION

Expression of *mai-1* and *gpd-2* under heat-shock control

We have previously described an *in vivo* system to study operon processing using a construct, HS1496,

derived from a three gene operon, *mai-1*, *gpd-2*, and *gpd-3* (Spieth et al., 1993). This construct contains *gpd-2* and *gpd-3* under control of the *hsp-16-41* heat-shock promoter inserted within the last exon of *mai-1*. In the current study, a 774-bp fragment containing the last two exons of *mai-1* encompassing 46 bp of 5' UTR and all of the *mai-1* coding region was cloned into a slightly modified HS1496 (see Materials and Methods) to create pGPDmaiWT (Fig. 1). This recreates most of the operon under control of the heat-shock promoter, but with a presumably inconsequential 8 bp change in the *mai-1* 3' UTR 38 bp upstream of the 3' end. The construct was introduced into worms to generate transgenic strains. The strains were subjected to a standard heat shock regimen and RNA was extracted and tested by both RNase protection analysis (RPA) and primer extension (PE) in the presence of either ddGTP or ddCTP to analyze polycistronic pre-mRNA processing.

Mai-1 3'-end formation

To measure 3'-end formation of *mai-1*, we used an RPA probe that extends from 219 bp upstream of the proximal *mai-1* 3'-end formation site to 17 bp beyond the *gpd-2* *trans*-splice site (Fig. 1). With this probe, we can see products protected by unprocessed RNA (351 nt), and two different cleaved and polyadenylated RNAs (333 nt and 219 nt). *mai-1* 3' end formation occurs at two sites: a proximal site 13–16 nt downstream of the AGUAAA, and a distal site an additional 115 bp downstream, right at the *gpd-2* *trans*-splice site (Spieth et al., 1993; Fig. 1). Figure 2, lanes 2–4, shows a prominent RPA product of about 330 nt, indicating that most 3'-end formation occurs at the distal site. A barely detectable product of about 220 nt indicates that a very

FIGURE 1. The *mai-1/gpd-2/gpd-3* operon. The *C. elegans* *hsp-16-41* promoter upstream of *mai-1* (triangle) drives expression of *mai-1*, *gpd-2*, and *gpd-3/vit-6*. Filled bars: coding regions; open bars: noncoding regions of exons; narrow lines: introns; wider lines: intercistronic sequences. The RPA probe (thick bar above the gene diagram) extends from within the last exon of *mai-1* to +17 of *gpd-2*. The intercistronic sequence between *mai-1* and *gpd-2* is shown below. The sequence is numbered from the *mai-1* proximal 3' end site. The 3' end sites of *mai-1* and the *trans*-splice site of *gpd-2* are indicated. The *mai-1* AGUAAA 3'-end formation signal is boxed and the *gpd-2* *trans*-splice site is underlined.

FIGURE 2. Effect of the AAUAAA mutation on 3'-end formation. Thirteen transgenic strains carrying the indicated constructs, along with a *smg-2* nontransgenic control strain, were grown and heat shocked. RNA was isolated and subjected to RPA with the probe extending from 219 bp upstream of the *mai-1* proximal 3' end to 17 bp 3' of the *gpd-2* trans-splice site. Lanes are labeled with the sequence at the 3' end formation signal site (above) and whether or not the trans-splice site was present (+) or absent (-). Positions expected for expected protected products are indicated.

small percentage of *mai-1* 3'-end formation occurs at the proximal site immediately following the AGUAAA. The presence of a small amount of polycistronic RNA resulting from failure of 3'-end formation indicates that cleavage dependent on AGUAAA is inefficient.

To test the idea that the low accumulation of *mai-1* mRNA is due to the rare AGUAAA 3'-end formation signal, we changed 1 bp to create the consensus AAUAAA sequence. Transgenic worm strains carrying this construct were generated, and total RNA was extracted and tested as above by RPA. This mutation had a profound effect (Fig. 2, lanes 11 and 12): first, no polycistronic RNA accumulated, suggesting that 3'-end formation is more efficient; second, the site of 3'-end formation was altered such that the site proximal to the AAUAAA was used almost exclusively; and third, far more *mai-1* 3' end product accumulated, suggesting that the efficient cleavage and polyadenylation directed by the perfect AAUAAA may prevent degradation of the *mai-1* mRNA that has been cleaved by trans-splicing but not yet polyadenylated.

Requirements for 3'-end formation at the distal site

The distal 3' end formation site is intriguing for two reasons: (1) it occurs immediately adjacent to the trans-splice site, so it is possible that it results from trans-splicing-dependent cleavage; and (2) there is no CPSF binding site close to it, although the AGUAAA 115 nt upstream could be functioning at this distant site. To test these ideas, we created two mutations. We replaced the AGUAAA with unrelated sequence (AUC

GAU), and we eliminated the trans-splice site (UUUCAG to GGUAC) in the context of the wild-type AGUAAA and the mutant AAUAAA 3'-end formation sites. RNA from two or three transgenic strains containing each construct was assayed for 3'-end formation. The data clearly show that the AGUAAA is not required for use of the distal site, as worms carrying the AUCGAU mutant construct can still form 3' ends at the distal site (Fig. 2, lanes 5–7). In contrast, the trans-splice site is required for distal 3'-end formation, as mutation of the trans-splice site eliminated all 3'-end formation at the distal site and resulted in accumulation of high levels of unprocessed pre-mRNA instead (Fig. 2, lanes 8–10 and 13–14). We conclude that distal 3'-end formation requires the trans-splice site, but not an AAUAAA or one of its close relatives.

Trans-splicing of *gpd-2*

To determine whether the *gpd-2* mRNA is trans-spliced to SL1 or SL2, we performed PE in the presence of either ddGTP or ddCTP (Kuersten et al., 1997). These assays distinguish between SL1 and SL2 trans-spliced mRNA due to differences in the sequences upstream of the trans-splice site. In the presence of ddGTP, reverse transcription from a primer that anneals to the trans-splice site terminates at the first C, giving products that extend 9 nt with SL1-spliced mRNA, 3 nt with SL2-spliced mRNA, or 2 nt with pre-mRNA or other unspliced intermediates. In the presence of ddCTP, the corresponding extensions are 2 nt for SL1, 9 nt for SL2, or 13 nt for unspliced pre-mRNA. As can be seen in Figure 3, lanes 2–4, in the case of the wild-type construct, the vast majority of *gpd-2* mRNA was trans-spliced to SL1, with only a small amount (~1%) of SL2 trans-spliced product.

When the *mai-1* AGUAAA was changed to AAUAAA, although a substantial level of SL1 trans-splicing still occurred, a significant proportion (~9%) of the *gpd-2* trans-splicing was to SL2 (Fig. 3, lanes 8–9). This observation complements our previous finding that elimination of the *gpd-2* 3'-end formation signal resulted in reduction of SL2 trans-splicing of *gpd-3* (Kuersten et al., 1997). We also tested the effect of elimination of the AAUAAA in the *mai-1* operon context (Fig. 3, lanes 5–7). In this case, we found that elimination of the CPSF binding site resulted in elimination of SL2 trans-splicing. These observations lead us to conclude that either the efficiency of 3'-end formation of the upstream gene or simply the presence of CPSF influences the specificity of trans-splicing of the gene just downstream.

Implications for the mechanism of 3'-end formation in *C. elegans*

Unlike in vertebrates, quite a few variants of the AAUAAA consensus are utilized as 3'-end formation

FIGURE 3. Increase in SL2 *trans*-splicing by the AAUAAA mutation. RNA samples from transgenic strains and from the *smg-2* control strain were tested by primer extension from a primer that extends from position -1 from the *gpd-2* *trans*-splice site in the presence of either ddCTP or ddGTP. Following primer extension, the products were electrophoresed on a 20% polyacrylamide denaturing gel. Positions of expected products are indicated.

signals in worms, presumably as CPSF binding sites (Blumenthal & Steward, 1997). This could be a way of varying the efficiency of 3' end formation and hence the resulting level of the mRNA. Alternatively, the rarer variants could be compensated for by a tighter CstF binding site so that the overall efficiency of 3'-end formation would be relatively constant but with different RNA/protein interactions providing the majority of the binding energy. However, at this time, nothing is known about CstF binding sites in *C. elegans* (although genes for all three CstF subunits are present in the genome (C. Williams and T. Blumenthal, unpubl. observations).

The data unequivocally demonstrate that a single base mismatch to the AAUAAA consensus can result in a lower efficiency of 3'-end formation, so it is likely that in *C. elegans* 3'-end formation frequency and therefore mRNA abundance is controlled by the quality of match to the AAUAAA consensus. We hypothesize that there is a direct correlation between the frequency of use of the various acceptable signals and the strength of the resulting 3'-end formation site. The *mai-1* CPSF site, AGUAAA, is a very rare one (Blumenthal & Steward, 1997) and is therefore likely to represent a relatively weak signal. Moreover, *C. briggsae* has the same sequence at this position, suggesting its functional importance (Lee et al., 1992). Indeed, we have shown here that changing it to the consensus sequence makes 3'-end formation at the proximal site much more frequent

and thereby increases the level of total *mai-1* mRNA and of the shorter 3' end in particular. The significance of this is not clear, although it is worth noting that it was somewhat difficult to obtain transformants containing this construct, possibly due to overexpression of MAI-1 resulting from the increased level of *mai-1* mRNA with the proximal 3' end. *mai-1* encodes the mitochondrial ATPase inhibitor, a subunit of the ATP synthetase, a protein whose level could be quite important in balancing cellular energy metabolism.

In the absence of a close match to the AAUAAA sequence, *mai-1* 3' ends are formed at a distal site that is at or near the site at which *trans*-splicing cleaves. This is intriguing because it suggests a novel mechanism of 3'-end formation involving cleavage by *trans*-splicing, followed presumably by debranching and by polyadenylation. This idea is strongly supported by our demonstration that mRNA polyadenylated at the distal site is dependent on the *trans*-splice site. mRNA formed at the distal site is entirely replaced by unprocessed RNA when the *trans*-splice site is mutated.

If cleavage at the distal site occurs by *trans*-splicing, what signal results in polyadenylation? A close inspection of the sequence of the *mai-1/gpd-2* intercistronic region reveals no sequence related to AAUAAA other than the AGUAAA studied here. This suggested the possibility that the AGUAAA could have been functioning to allow polyadenylation 130 nt downstream, at the

site of *trans*-splicing. Indeed, in vitro experiments with mammalian 3'-end formation have demonstrated that CPSF is capable of acting at a distance of >400 nt to allow polyadenylation of a precleaved substrate (Manley et al., 1985). However, we found that distal 3'-end formation was not dependent on the AGUAAA, because the level of distal product was not changed by deleting this sequence. We do not know how the RNA gets polyadenylated at the distal site, but it is worth noting that a substantial number of *C. elegans* 3'-end formation sites do not have any recognizable match to the AAUAAA consensus (Blumenthal & Steward, 1997). Perhaps there is another way to attract the CPSF/CstF complex to the pre-mRNA in *C. elegans*.

Role of the AAUAAA in downstream *trans*-splicing

We have shown previously that mutation of the AAUAAA at the 3' end of *gpd-2* alters the *trans*-splicing specificity of *gpd-3*; *trans*-splicing by SL2 was decreased whereas SL1 was increased (Kuersten et al., 1997). However, it was not clear whether the effect was direct or indirect. The 3' end formation machinery could play a direct role in facilitating SL2 *trans*-splicing, or cleavage could create a substrate on which SL2 *trans*-splicing is favored. It is even possible that SL1 *trans*-splicing is favored on the polycistronic substrate, so cleavage results in SL2 *trans*-splicing by eliminating the substrate for SL1. Here we have presented the converse experiment: efficient 3'-end formation dependent on an optimal CPSF binding site increases SL2 *trans*-splicing in a situation in which it is normally infrequent. However, it is worth noting that even when the consensus AAUAAA is present in this operon, most *trans*-splicing is nevertheless to the SL1 leader. Thus it is clear that the closeness of the match to the AAUAAA is not the sole attribute that defines whether the *trans*-splicing event involves the SL1 or SL2 snRNP.

Our laboratory recently characterized a novel class of operon in which the two genes are immediately adjacent, with no base pairs between the 3'-end formation site of the upstream gene and the *trans*-splice site of the downstream gene (Williams et al., 1999). In this rare class of operon, *trans*-splicing of the downstream gene is to SL1 exclusively. Because there are only a few base pairs between the AAUAAA and the *trans*-splice site consensus sequence, 3'-end formation and *trans*-splicing occur at adjacent nucleotides. The observations made here are consistent with the idea that, in many ways, the *mai-1/gpd-2* gene pair is behaving like an SL1-type operon: Most of the 3' ends are formed at the *trans*-splice site and most of the *trans*-splicing is to SL1. The main difference seems to be that there is a weak 3' end signal 130 nt upstream, instead of a perfect AAUAAA right at the *trans*-splice site. The result is that a small fraction of the upstream product has its 3'

end at a site typical of SL2-type operons and approximately an equivalent fraction of the downstream product is *trans*-spliced to SL2. When we convert the AGUAAA to AAUAAA, the operon behaves more like a typical one; most of the 3' ends form at the proximal site, 115 bp upstream of the *trans*-splice site, and much of the *trans*-splicing is to SL2.

MATERIALS AND METHODS

Worm strains and RNA preparation

Maintenance and growth of worms was as described previously (Brenner, 1974; Sulston & Hodgkin, 1988). Transgenic worm strains carrying extrachromosomal arrays were generated by injection of plasmid DNA mixtures into strain TR1332 *smg-2* (r863) worms as described previously (Mello et al., 1991; Spieth et al., 1993). Worms were heat shocked at 29 °C for 1–2 h on floating petri plates spread with bacteria. Total RNA was prepared from heat-shocked mixed-stage populations of transgenic worms (Conrad et al., 1991).

Plasmid construction

Most of the *mai-1* gene was inserted in its original position as the first gene in the *gpd-2/3* operon, to create pGPD*mai*WT. This plasmid was constructed from HS1496 (Spieth et al., 1993), which contains the *gpd-2* and *gpd-3* genes under the control of the *hsp-16* promoter, cleaved with *Sph*I, followed by deletion of 153 bp by limited *Ba*l31 digestion. (This modification of nonessential vector sequences was made to delete one of the two *Sa*I sites. However, the resulting unique *Sa*I site was not used for any of the constructs utilized in this paper.) Part of the *mai-1* gene containing the second and third exons was amplified by PCR from *C. elegans* genomic DNA using the following *Bam*HI site-containing primers: TTG GATCCATGGTCATATAATCCAGTTAG and AAGGATCCGC GAAAAAAAAATGATGC. The resulting PCR fragment was digested with *Bam*HI and cloned into the *Bam*HI site of HS1496 containing the 153 bp deletion.

The plasmid pGPD*mai*pA was derived from pGPD*mai*WT by replacing a single base pair in the AGTAAA 3'-end formation signal of *mai-1* to create AATAAA via recombinant PCR (Higuchi, 1990). The primers at the ends were CTTATCTGC GAAAGTGGC and CCCATTGGATTTCAGCTGGG. The following pairs of primers were used to introduce the mutation: TCGTCAAATAATAAACATGGTTTTTATGGC and ATGTTTATTATTTGACGAAACATGGG.

The plasmids pGPD*mai*ΔpA and pGPD*mai*ΔTS were derived from pGPD*mai*WT as follows: pGPD*mai*WT was cut with *Pst*I and *Stu*I to excise a fragment containing part of *mai-1* and *gpd-2*, which was cloned into the polylinker of pLIT (New England Biolabs) cut with the same enzymes. To make pGPD*mai*ΔpA, the polyadenylation site (AGTAAA) was replaced by a *Cl*aI restriction site (ATCGAT) by run-around PCR (Coolidge & Patton, 1995) using the following oligonucleotides: GATCATGGTTTTTATGGCTTCAC and GATA TTTGACGAAACATGGGGG. To make pGPD*mai*ΔTS, the *trans*-splice site (TTTCAG) was replaced with a sequence (GGUAC) to create a *Kpn*I restriction site by run-around PCR

using the following oligonucleotides: GGTACCCATGCCAA AGCCAAATG and ATTGATACTACTTACAATCAGTTC. The *Pst*I/*Stu*I fragments containing the desired mutations were then used to replace the homologous fragment of pGPDmaiWT.

The plasmid pGPDmaipAΔTS was made as described above for pGPDmaiΔTS, but using pGPDmaipA as template. All plasmids were sequenced to confirm their identities.

RNase protection assay and primer extension analysis

The RPA probe was made by PCR amplification of the region of *mai-1* and *gpd-2* shown in Figure 1 using each of the plasmids as templates. The primers were AAATCGAGAAAG GACACGG and ATTTGGCTTTGGCATGGC. PCR products were cloned into pGEM T-easy vector system I (Promega). The plasmids were transcribed (Ampliscribe kit, Epicentre) with ³²P-UTP to make RPA probes. The protocol used for RPA has been described previously (Kuersten et al., 1997). Primer extension in the presence of dideoxynucleotides was performed as described previously (Kuersten et al., 1997) using ATTTGGCTTTGGCATGGC as the primer.

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REFERENCES

- Blumenthal T, Steward K. 1997. RNA processing and gene structure. In: Riddle D, Blumenthal T, Meyer B, Priess J, eds. *C. elegans II*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 117–145.
- Brenner S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77:71–94.
- Conrad RC, Thomas J, Spieth J, Blumenthal T. 1991. Insertion of part of an intron into the 5' untranslated region of a *C. elegans* gene converts it into a *trans*-spliced gene. *Mol Cell Biol* 11:1921–1926.
- Coolidge CJ, Patton JG. 1995. Run-around PCR: A novel way to create duplications using polymerase chain reaction. *Biotechniques* 18:762–764.
- Higuchi R. 1990. Recombinant PCR. In: Innis MA, Gelfand DH, Sninsky JJ, White T, eds. *PCR Protocols: A guide for methods and applications*. San Diego, California: Academic Press, Inc. pp 177–183.
- Huang XY, Hirsh D. 1989. A second *trans*-spliced RNA leader sequence in the nematode *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 86:8640–8644.
- Kuersten S, Lea K, MacMorris M, Spieth J, Blumenthal T. 1997. Relationship between 3' end formation and SL2-specific *trans*-splicing in polycistronic *Caenorhabditis elegans* pre-mRNA processing. *RNA* 3:269–278.
- Lee YH, Huang X-Y, Hirsh D, Fox GE, Hecht R. 1992. Conservation of gene organization and *trans*-splicing in the glyceraldehyde-3-phosphate dehydrogenase-encoding genes of *Caenorhabditis briggsae*. *Gene* 121:227–235.
- Manley JL, Yu H, Ryner L. 1985. RNA sequence containing hexanucleotide AAUAAA directs efficient mRNA polyadenylation in vitro. *Mol Cell Biol* 5:373–379.
- Mello C, Kramer J, Stinchcomb D, Ambros V. 1991. Efficient gene transfer in *C. elegans*: Extrachromosomal maintenance and integration of transforming sequences. *EMBO J* 10:3959–3970.
- Spieth J, Brooke G, Kuersten S, Lea K, Blumenthal T. 1993. Operons in *C. elegans*: Polycistronic mRNA precursors are processed by *trans*-splicing of SL2 to downstream coding regions. *Cell* 73:521–532.
- Sulston J, Hodgkin J. 1988. Methods. In: Wood W, ed. *The nematode Caenorhabditis elegans*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 587–606.
- Williams C, Xu L, Blumenthal T. 1999. SL1 *trans*-splicing and 3' end formation in a unique class of *Caenorhabditis elegans* operon. *Mol Cell Biol* 19:376–383.
- Zhao J, Hyman L, Moore C. 1999. Formation of mRNA 3' ends in eukaryotes: Mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *Microbiol and Mol Biol Rev* 63:405–445.
- Zorio DAR, Cheng NN, Blumenthal T, Spieth J. 1994. Operons represent a common form of chromosomal organization in *C. elegans*. *Nature* (London) 372:270–272.