***C. elegans* mRNAs acquire a spliced leader through a trans-splicing mechanism**

S.L.Bektesh and D.I.Hirsh

Development Biology, Synergen, Inc., 1885 33rd Street, Boulder, CO 80301, USA
Submitted May 10, 1988

A 22 nt spliced leader (SL) is added to some *C. elegans* mRNAs by a process of discontinuous RNA synthesis (1). The SL is synthesized as part of a larger precursor RNA, the leader RNA (LR) which is about 100 nt long. The LR has a consensus splice donor sequence following the 22 nucleotide SL sequence. In the genomic sequences of mRNAs that acquire an SL, there is a consensus splice acceptor site adjacent to the splice junction. The conservation of sequences necessary for intron splicing led us to believe that the two most likely mechanisms for the acquisition of the SL are (a) ligation of the precursor RNAs followed by conventional cis-splicing (Fig. 1A) and (b) trans-splicing (Fig. 1B). A prediction from trans-splicing is a Y-branched intermediate that releases a 78 nt RNA (nt 23 through 100 of the LR) upon incubation with debranching enzyme (2,3).

We incubated total *C. elegans* RNA either with or without debranching enzyme (4), then probed an electroblot Northern with an oligonucleotide complementary to nt 69 through 87 of the LR. The results are shown in Fig. 2. In the absence of debranching enzyme, the probe hybridizes primarily to the LR of 100 nt. There is a smear of hybridization to higher molecular weight species (>500 nt) which we propose may be splicing intermediates. However, incubation of total RNA with debranching enzyme yields a discrete RNA of about 78 nt. These results are consistent with the involvement of a Y-branched intermediate in trans-splicing.

Fig. 1. Possible Mechanisms for SL Addition

Fig. 2. Treatment of *C. elegans* Total RNA with Debranching Enzyme

Acknowledgements: We thank J. Arenas and J. Hurwitz for a gift of debranching enzyme. This work was supported by a grant from the U.S. Public Health Service, No. GM37823.

References: 1. Krause, M., Hirsh, D. (1987) *Cell* **49**, 753-761. 2. Murphy, W.J. et al. (1986) *Cell* **47**, 517-525. 3. Sutton, R.E., Boothroyd, J.C. (1986) *Cell* **47**, 527-535. 4. Arenas, J., Hurwitz, J. (1987) *J. Biol. Chem.* **262**, 4274-4279.