We also carried out experiments to determine whether RNAs synthesized from separate plasmids could be *trans*-spliced *in vitro*. SL RNA and actin-1 pre-mRNAs were separately synthesized *in vitro*, gel-purified and then added either alone or in combination to a HeLa cell nuclear extract. The products were then selected with the *C. elegans* spliced-leader-specific 2′-OCH_3 oligonucleotide and assayed by RNase protection (Fig. 2). When SL RNA alone was added to the extract, a short protected fragment corresponding to spliced leader exon was observed (lane 3). Similarly, a protected RNA fragment corresponding in size to unspliced RNA was oberved when *in vitro*-synthesized actin pre-mRNA was added to the extract (lane 4). But, like the situation *in vivo*, a protected band corresponding to the size of *trans*-spliced product was observed when both the SL RNA and 3′ *trans*-splicing acceptor were added to the *in vitro* reaction (lane 5). The DNA sequence of the RT-PCR products with both *C. elegans* and *L. collosoma* SL RNAs and the actin-1 acceptor from *in vitro* reactions confirmed the generation of accurately *trans*-spliced products (data not shown). These results argue against the possibility that the *trans*-splicing observed *in vivo* was due to a low level of recombination between the cotransfected plasmids followed by *cis* splicing. They also establish the feasibility of studying *trans*-splicing in mammalian extracts.

Previous studies demonstrated that the nematode SL RNA can be spliced to an adenovirus pre-mRNA 3′ splice site *in vitro*, when the two splice sites are present in the same RNA (in *cis*)[15]. To determine whether this mammalian 3′ splice site can also participate in *trans*-splicing, the SL RNA expression vector was cotransfected with a vector containing the adenovirus 3′ splice site. As with the nematode 3′ splice site, *trans*-spliced product was observed only when the two plasmids were cotransfected (data not shown). The DNA sequence of the RT-PCR product from 2′-OCH_3-selected RNA revealed that the SL RNA is accurately *trans*-spliced to the adenovirus 3′ splice site (Fig. 3).

The demonstration of *trans*-splicing of nematode and trypanosomatid SL RNAs in mammalian cells provides an additional evolutionary link between *cis*- and *trans*-splicing[16], and splicing in lower and higher eukaryotes. All of these splicing reactions, as well as the self-splicing of group II introns, proceed through the same two-step mechanism, involving the formation of a 2′-5′ branched intermediate and product[17-20]. In group II introns, all of the machinery necessary for splicing is contained within the intron, whereas pre-mRNA splicing takes place in spliceosomes, complex particles containing small nuclear ribonucleoproteins (sRNPs) and a large number of non-snRNP splicing factors[21,22]. *Trans*-splicing seems to represent an intermediate evolutionary stage in which both the 5′ exon and an snRNP-like region are contained within the SL RNA. If the functions of the U1 and U5 snRNPs are to recognize intron and exon sequences, respectively, at both the 5′ and 3′ splice sites[23-25], then splice site recognition in *trans*-splicing may involve analogous activities within the SL RNA[25].

We have shown that accurately initiated and terminated SL RNAs are assembled into SL snRNPs in mammalian cells, and that a nematode 3′ *trans* acceptor, as well as the normally *cis*-spliced adenovirus 3′ splice site, are assembled into splicing complexes capable of functional interactions with SL RNAs. *In vitro* spliceosome assembly studies in mammalian cell extracts have shown that pre-spliceosome complexes containing specific splicing components can assemble on pre-mRNAs containing only a 5′ or 3′ splice site[26,27]. In the *trans*-splicing reaction, complexes assembled on separate RNA molecules may interact by virtue of the utilization of novel splicing factors or unique interactions of known splicing components. For example, it has been shown that the nematode SL RNA interacts with the U6 snRNP (T. Nilsen, personal communication) in a region of U6 previously seen to base-pair with the U2 snRNP in mammalian splicing systems[28-30].

In summary, we have shown that the mammalian splicing apparatus can carry out *trans*-splicing by a mechanism involving SL RNAs. We are currently exploiting this observation to search for mammalian SL RNAs and *trans*-splicing acceptors. □

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CORRECTION

Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*

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EXAMINATION of the sequence for a homologue of the polypeptide described in the above paper (EMBL accession number X62160) raised the possibility that a small segment of the rat sequence as originally reported was in an incorrect translational frame. We have closely examined the original data and conclude that two sequencing errors caused the reading frame for nucleotides 1,099-1,214 to be shifted by one base. The corrected form predicts an amino-acid sequence for residues 277-315 of EAKEAKEALEAKERYMEEMADTADAIEMATLDKEMAEER.

The revised sequence now shows an even greater extent of homology with the predicted polypeptide product of the *Drosophila* gene *Glued*, as well as a greater extent of coiled-coil alpha helix consistent with the structure of the *Glued* gene product. Therefore none of the conclusions of the paper is altered. As a clearer designation for the rat polypeptide which we characterized, we now suggest the name $p150^{Glued}$. The revised sequence has been submitted to EMBL.