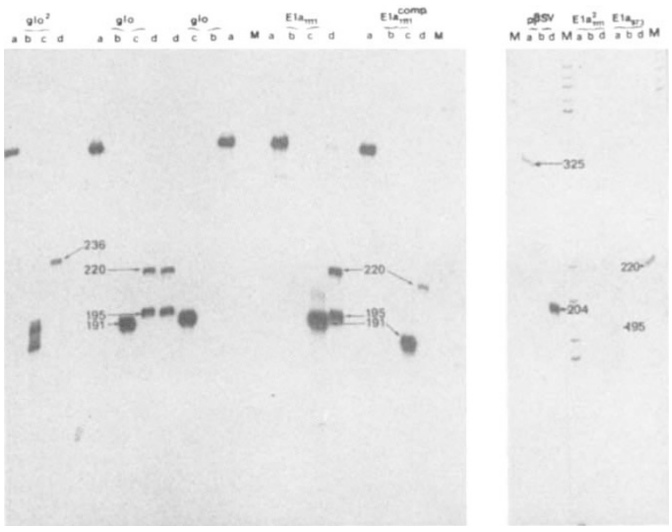

Fig. 3 Determination of the splice sites chosen using S_1 nuclease, for β-globin genes containing the following test sequences: rabbit β-globin IVS-2 5′ splice site (glo), adenovirus 2 *E1a* sites 973 and 1111 ($E1a_{973}$ and $E1a_{1111}$), a tandem dimer of $E1a_{1111}$ ($E1a^2_{1111}$) and a complementary insert ($E1a^{comp}_{1111}$), and no insert ($p\beta SV$). Lanes a, b, c, d and M are as described for Fig. 2. Numbers refer to sizes in base pairs. Samples were run on two 5% sequencing gels, containing 20% formamide; RNA was hybridized to the probe overnight at 49 °C and standard conditions were used for S_1 nuclease digestions[14]. The two sets of lanes for glo represent independent isolates.

(4) The sequences of the cryptic 5′ splice sites are not used; thus, the fact that these sites are not used in the β-globin gene (unless the normal IVS-2 5′ splice site is not available[4]) can be attributed to their sequence without invoking effects of secondary structure or position.

(5) The results from constructions glo^2 and glo^4 illustrate the importance of secondary structures. Short inverted repeats can sequester a site and cause splicing to occur only at the reference. This is a new way in which secondary structure can influence splicing, involving the site directly. It has been shown[1] that an exon flanked by inverted repeats can be sequestered such that it is excised within the intron. It is possible that the effect of secondary structure would be minimized if no choice was available, and we are testing this by inactivating the reference site in glo^2.

(6) Another factor which affects the choice of sites is that of relative position. If we assume that the different flanking sequences of the two signals in the Ela^2_{1111} tandem dimer have no bearing on their use, it seems that the upstream site is used preferentially and use of the reference site is drastically reduced. This construction gave rise to a comparatively faint band on S_1 analysis (Fig. 3); whether this is significant can be resolved only by experiments *in vitro*. The significance of linear order may be influenced by the distance between two sites or by their environment; when a single β-globin sequence is inserted it is preferred to the reference β-globin site by a ratio of only 2:1.

(7) The two *E1a* splice site sequences are used with very different efficiencies, even though both sites are used when intact *E1a* genes are expressed in HeLa cells. Experiments in which β-globin restriction fragments were duplicated[2] showed that an upstream site is usually, but not always, used to the exclusion of a downstream site. The results described in (6) emphasize the linear 5′ to 3′ order of alternative sites. Thus, we anticipated that in the *E1a* gene a much more efficient sequence might be found at the downstream site in order to balance its unfavourable position. This prediction was shown to be correct (Table 1, and unpublished results with sites intermediate in efficiency between glo and cryptic sites in our assay), but further experiments are required to test the importance of linear order and pre-mRNA structure.

(8) The fact that splicing can be directed to the inserted oligonucleotide, away from a viable and efficient natural site, has mechanistic consequences. If two 5′ splice site-binding complexes[8,9] bind simultaneously to the two sites, it is unlikely that the two sites could still be distinguished on the basis of their sequences in subsequent steps. However, we have shown here that primary sequence alone is the basis of discrimination. It is possible that both 5′ sites independently bind a complex and that the ratios of site usage are related to the average lifetime of bound complex. We propose a model in which only one 5′ splice site complex can bind, perhaps limited by a requirement for the presence of a 3′ site complex (in agreement with some[8,10] but not all[9] *in vitro* data). The 5′ splice site complex can bind to either 5′ site, but the dissociation rates differ and the resulting pattern of usage then depends on the lifetime at each site. The involvement of only one 5′ complex is rendered very likely by the proximity of our sites; experiments that increase the separation between the two 5′ splice sites will reveal whether the sequence discrimination and the model only apply to such close sites.

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Errata

Generation of single-stranded T-DNA molecules during the initial stages of T-DNA transfer from Agrobacterium tumefaciens to plant cells

S. E. Stachel, B. Timmerman & P. Zambryski
Nature **322**, 706-712
In this article, the title given on the contents page was incorrect. The title is correct as above.

A molecular link between the bats of New Zealand and South America

E. D. Pierson, V. M. Sarich, J. M. Lowenstein, M. J. Daniel & W. E. Rainey
Nature **323**, 60-63
In this letter, a line was omitted from the legend for Table 1, and *yumanensis* was misspelt. The correct version reads:

The taxa used in these comparisons represent all the families with which *Mystacina* has been associated: *noctilio albiventris* (Noc) (Noctilionidae); *Mormoops megalophylla* (Mor) and *Pteronotus parnllii* (Moemoopidae); *Glossophaga soricina* (Glo) and *Phyllostomus discolor* (Phyllostomidae); *Antrozous pallidus* (Ant) and *Myotis yumanensis* (Vespertilionidae); *Tadarida brasiliensis* (Tad) (Molossidae); *Balantiopteryx plicata* (Bal) (Emballonuridae).