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Aberrant splicing of *Drosophila* alcohol dehydrogenase transcripts in *Saccharomyces cerevisiae*

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We have investigated the ability of transcripts of the *Drosophila melanogaster* alcohol dehydrogenase gene to be spliced in *Saccharomyces cerevisiae*. The alcohol dehydrogenase gene was cloned in *S. cerevisiae* on a 2 micron DNA-based vector and a hybrid yeast actin-*Drosophila* alcohol dehydrogenase gene was constructed to demonstrate that transcripts encoded on a 2 micron plasmid could be accurately and efficiently spliced. Transcription of the *Drosophila* gene occurred in yeast with and without a yeast promoter. The transcripts were polyadenylated and terminated ~600 nucleotides distal to the polyadenylation site used in *Drosophila*. In yeast no splicing of the two introns within the alcohol dehydrogenase coding sequence was detected. However, the leader sequence was apparently spliced using the same 3′ splice site as is used in adult flies, but a different 5′ splice site. This result may be partly explained by the existence in the *Drosophila* gene of a sequence which is believed to be required for splicing in *S. cerevisiae*.

Key words: alcohol dehydrogenase/introns/splicing/yeast

Introduction

Splicing of RNAs to remove intervening sequences is widespread among eukaryotes, occurring in nuclear transcripts produced by polymerases I, II and III and in mitochondria (for reviews, see Lewin, 1982; Breathnach and Chambon, 1981; Abelson, 1979; Dujon, 1981). The splicing of each of these types of transcripts occurs by different mechanisms and, although RNA splicing was first observed with polymerase II transcripts, very little is known about how this process occurs.

The most striking features of introns in nuclear protein-coding transcripts are the conserved sequences at the 5′ and 3′ ends (Breathnach and Chambon, 1981; Mount, 1982). The existence of these conserved sequences suggests that the machinery for splicing protein-coding transcripts evolved from a common origin and may be relatively highly conserved among diverse eukaryotes. Indeed the introns from various genes of avian and mammalian origin can be accurately spliced from transcripts produced in heterologous tissue culture cells (Breathnach and Chambon, 1981). In addition, in monkey cells, a transcript of a chimaeric SV40-mouse β-globin gene containing a hybrid intron is spliced, using the donor site from SV40 and acceptor site from β-globin (Chu and Sharp, 1981).

If yeast contained a splicing activity for polymerase II transcripts compatible with that in higher eukaryotes, the functional expression of heterologous intron-containing genes in yeast would be feasible. However, Beggs *et al.* (1980) found that transcripts of a cloned rabbit β-globin gene were not spliced in *Saccharomyces cerevisiae*. These transcripts were incorrectly initiated, being 20–40 nucleotides shorter at the 5′ end than normal β-globin mRNA, terminated prematurely at T-rich sequences within the second intron, and were not spliced. Although the transcripts were polyadenylated, there was no evidence that they were products of polymerase II transcription. These considerations and the fact that the β-globin gene was introduced into yeast on a replicating 2 micron plasmid vector, the intracellular location of which was uncertain, qualified the conclusion that a heterologous transcript could not be spliced in yeast.

It was subsequently reported that the actin gene (Gallwitz and Sures, 1980; Ng and Abelson, 1980) and several nuclear ribosomal protein genes (Rosbash *et al.*, 1981; Bollen *et al.*, 1982; Leer *et al.*, 1982; Kaufer *et al.*, 1983) of yeast contain an intervening sequence with exon-intron boundary sequences resembling the eukaryotic consensus sequences. It therefore became possible to test the specificity of the yeast splicing machinery under more controlled conditions. This paper describes an analysis of the transcription of the *Drosophila melanogaster* alcohol dehydrogenase (ADH) gene and processing of the transcripts in *S. cerevisiae*.

ADH in *D. melanogaster* is coded by a single structural gene the expression of which is developmentally regulated, being maximal in larvae and in young adult flies. The transcripts produced in larvae and in adult flies differ in the position of the 5′ ends (Benyajati *et al.*, 1983), indicating regulation at the level of transcription by the differential use of two separate promoter regions. The transcripts also differ in the pattern of splicing depending on the stage of development. In addition to two introns within the coding sequence of each precursor, the primary transcripts in adult flies have a third intron in the leader sequence (Benyajati *et al.*, 1983; Figure 1a).

In this work transcription of the *Drosophila* ADH gene was directed in *S. cerevisiae* either from within the cloned *Drosophila* sequence or from the *S. cerevisiae* alcohol dehydrogenase I *(ADC)* promoter on a yeast 2 micron vector. Two of the three introns in the *Drosophila ADH* transcripts were not spliced in yeast, whereas the leader sequence was processed incorrectly compared with splicing in the adult fly. Evidence is presented for the efficient splicing of the yeast actin intron but not of a *Drosophila* intron in a hybrid transcript encoded on a 2 micron yeast vector, indicating that the aberrant splicing of transcripts of heterologous genes in yeast is a direct consequence of a difference in the specificity and/or mechanism of splicing in yeast. We discuss these results in the light of recent information on the sequence requirements for splicing in yeast.

Results

The *Drosophila* ADH gene was cloned as four different sized DNA restriction fragments (Figure 1a) into pMA56, a yeast expression vector containing the promoter for the yeast

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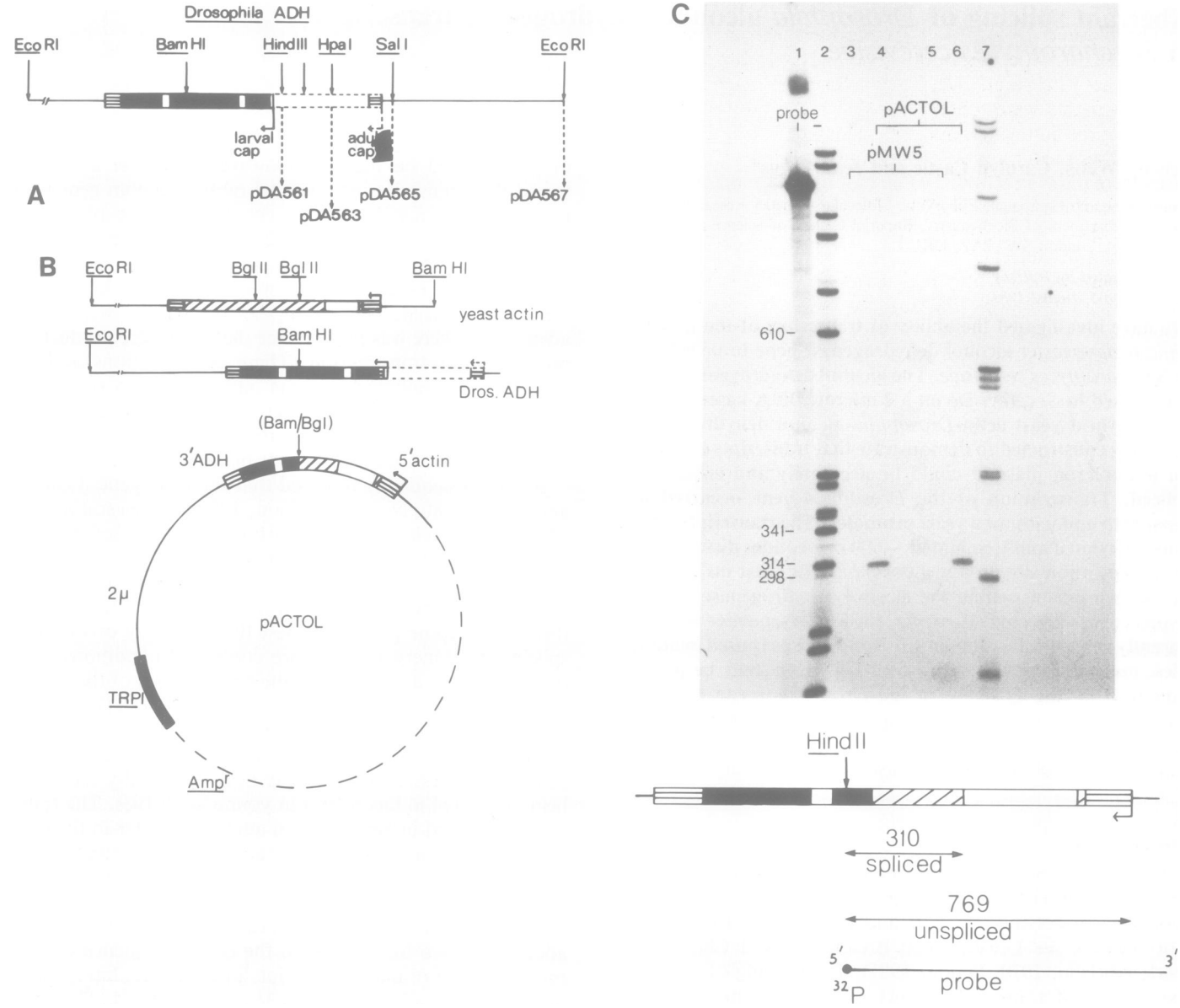

Fig. 1. A, A map of the region of the *D. melanogaster* genome including the ADH gene, indicating the extent of the fragments cloned in yeast vectors. The solid blocks represent coding sequences, the open blocks represent introns, and the striped blocks represent 5′ and 3′ untranslated regions of the transcripts. The positions of the 5′ ends or cap sites of transcripts produced in the adult fly and in larvae are indicated, with arrows showing the direction of transcription. The numbering of introns used in the text is 1 to 3 from right to left (i.e., from 5′ to 3′ in the transcripts), intron 1 being specific to the transcript from adult flies. Cloned fragments extend from the *Eco*RI site on the left to the position indicated by the corresponding plasmid number (pDA561 to 7), and were inserted at the *Eco*RI site of pMA56, using *Eco*RI linker fragments, in the appropriate orientation for transcription of the ADH gene from the yeast *ADC* promoter. The corresponding clones in pMW5 are denoted pMW51 to 57. **B,** Diagram indicating the composition of pACTOL. The top line shows the structure of the yeast actin gene, with diagonal stripes indicating the coding sequence, and horizontal stripes the 5′ and 3′ untranslated regions. The open box represents the intron, and the arrow shows the direction of transcription. The lower line shows the *Drosophila* ADH gene as in **A**. pACTOL was constructed by joining the *Bam*HI-*Bgl*II DNA fragment carrying the 5′ end of the yeast actin gene, to the *Bam*HI-*Eco*RI DNA fragment including the 3′ end of the *Drosophila* ADH gene and ligating to pMW5 DNA which had been digested with *Bam*HI and *Eco*RI. The construction of the hybrid gene ACTOL is such that the coding sequences of the yeast actin and the *Drosophila* ADH genes are not aligned, however there is an open reading frame through the fusion site to the second intron so that in theory the ACTOL transcript could be translated if both introns were accurately removed by splicing. **C,** S1 nuclease assay for splicing of the actin intron in pACTOL transcripts. The diagram shows the origin of the probe which was 5′-^{32}P-labelled at the *Hind*II site within the *Drosophila ADH* sequence and extended through the yeast actin sequence. RNA and DNA probe were annealed at 46°C and digested with 1000 units S1 nuclease/ml at 15°C. The protected fragments were detected by electrophoresis through a 3% polyacrylamide gel and autoradiography. The origin and length in nucleotides of probe fragments which would be protected by spliced or unspliced RNA are indicated in the diagram. **Lane 1,** undigested probe; **2** and **7,** DNA size markers; **3–6,** RNA from *S. cerevisiae* 302-21 transformed with pMW5 (**3** and **5**) or pACTOL (**4** and **6**).

ADH1 gene (*ADC*; Valenzuela *et al.*, 1982). The *Eco*RI-*Hind*III and *Eco*RI-*Hpa*I DNA restriction fragments (in pDA561 and pDA563) contain the complete coding sequence and the TATA sequence believed to direct transcription in larvae (Benyajati *et al.*, 1983), while the larger *Eco*RI-*Eco*RI and *Eco*RI-*Sal*I DNA restriction fragments (in pDA567 and pDA565, respectively) also include the entire leader sequence transcribed in the adult fly and the upstream TATA sequence. These two larger DNA fragments were also cloned in a related vector pMW5 (clones pMW57 and pMW55) which lacks the yeast *ADC* promoter, so that the effect of transcription from the yeast and *Drosophila* promoters could be com-

pared.

The plasmid DNAs were transformed into *S. cerevisiae* strain 302-21 which lacks all three yeast alcohol dehydrogenase activities, and *TRP*$^+$ transformants were selected. Transformants were tested for ADH activity by plating on complete medium containing 8% glucose and 1 μg antimycin A/ml. This medium prevents aerobic growth, and anaerobic growth requires functional ADH. All transformant clones as well as untransformed 302-21 cells were unable to grow, indicating that the *Drosophila* gene was unable to complement the yeast adh^0 defects.

Northern blots of poly(A)$^+$ RNA from the yeast clones were hybridised with *Drosophila ADH* purified DNA fragments as probes. All the *Drosophila* ADH plasmid constructs were transcribed in yeast (data not shown).

One possible reason for the lack of splicing of the rabbit β-globin transcripts in yeast (Beggs *et al.*, 1980) could be that transcripts of genes on a 2 micron plasmid vector are not spliced in yeast. To investigate this possibility a hybrid yeast actin-*Drosophila* ADH gene, ACTOL, was constructed (Figure 1b). This gene consists of the 5′ end of the yeast actin gene, including the actin promoter and intron, fused to the 3′ end of the *Drosophila* ADH gene including the third intron. The plasmid pACTOL is composed of the fused gene cloned in the yeast vector pMW5. pACTOL was introduced into *S. cerevisiae* strain 302-21 by transformation and shown by Southern blotting to be present in the replicating plasmid form, with no detectable copies integrated into high mol. wt. DNA (data not shown). Using a *Drosophila ADH*-specific probe, Northern blotting analysis of the poly(A)$^+$ RNA from a culture of 302-21 transformed with pACTOL detected a single transcript ~1500 nucleotides in length.

The result of S1 nuclease mapping of the actin intron of the hybrid transcripts is shown in Figure 1c. Poly(A)$^+$ RNA was annealed with a 1-kb *Hind*II-*Bam*HI fragment of the ACTOL gene which was 5′ end-labelled at the *Hind*II site. Since the *Hind*II site is in the *Drosophila* part of the gene, this probe is specific for hybrid transcripts and should not detect transcripts of the chromosomal actin gene. S1 nuclease digestion resulted in protection of 310 nucleotides of the DNA by RNA from cells transformed with pACTOL (Figure 1c, lanes 4 and 6). This is consistent with correct splicing of the actin intron from the hybrid transcript. The absence of any other bands in these tracks or in digests with RNA from cells transformed with the vector DNA containing no insert (Figure 1c, lanes 3 and 5) shows that the probe is specific for the hybrid transcript. This result demonstrates that transcripts of a gene located on a 2 micron plasmid can be accurately and efficiently spliced in yeast.

S1 nuclease mapping experiments were carried out to study the possibility of splicing of the *Drosophila ADH* transcripts. Figure 2 shows the results for intron 3 using a 2-kb *Bam*HI-*Eco*RI DNA restriction fragment of the *Drosophila* ADH gene 3′ end-labelled at the *Bam*HI site. As expected, protection of 89 nucleotides was observed using RNA from *Drosophila*, however RNA from yeast cells transformed with pDA561 did not protect a fragment of this size; instead protection of 1100 nucleotides was observed. This indicates that the third intron was not spliced out of transcripts produced in yeast and that transcription terminated ~600 nucleotides downstream from the poly(A) addition site used in *Drosophila*. The same result was obtained with transcripts of all the *ADH* constructs, including pACTOL (and is consistent with

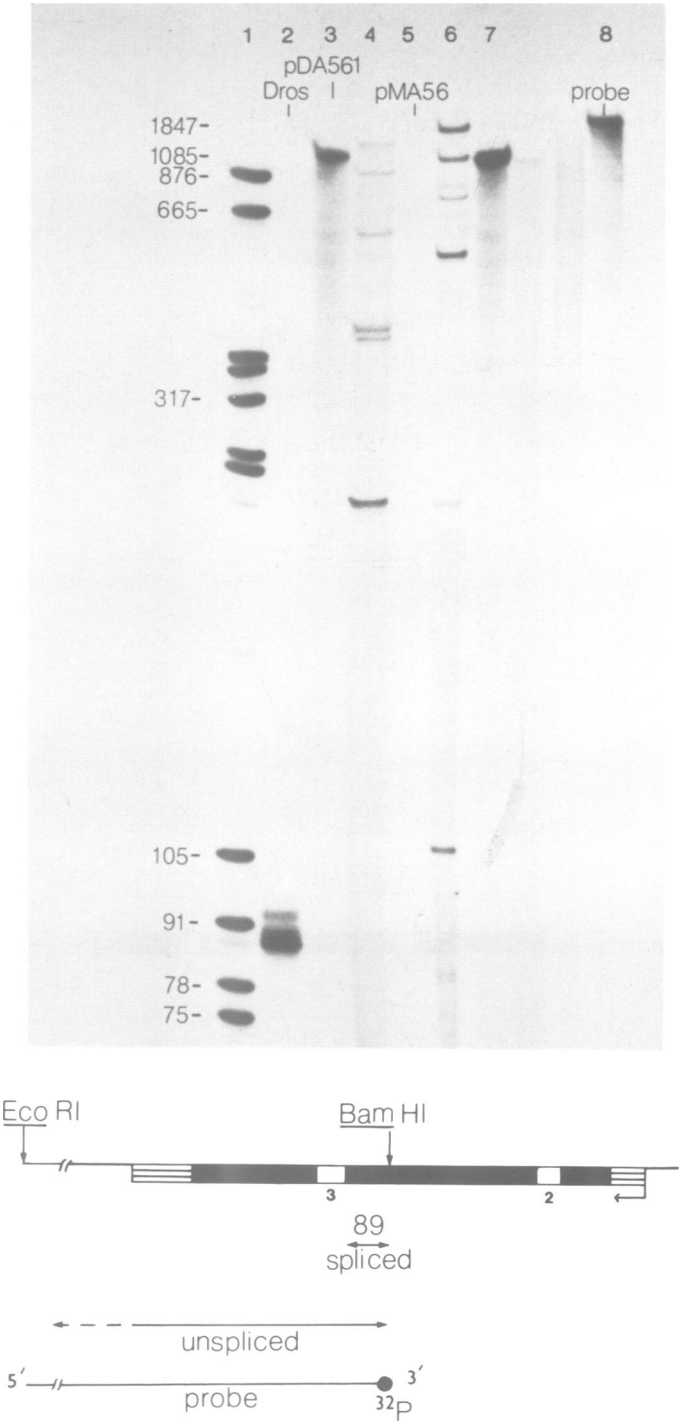

Fig. 2. S1 nuclease assay for splicing of *Drosophila ADH* intron 3. The probe was a 2-kb *Bam*HI-*Eco*RI fragment of the *Drosophila* ADH gene ^{32}P-labelled at the 3′ terminus of the *Bam*HI site. RNA and probe were annealed at 52°C and digested with 200 units S1 nuclease/ml at 15°C. The sizes of the protected fragments were estimated by polyacrylamide (5%) gel electrophoresis and autoradiography. The diagram indicates the origin of the probe and the protected fragments. **Lanes 1, 4** and **6**, DNA size markers; **2**, *Drosophila* poly(A)$^+$ RNA; **3** and **7**, RNA from *S. cerevisiae* strain 302-21 transformed by pDA561; **5**, RNA from strain 302-21 transformed by the vector, pMA56; **8**, undigested probe.

the 1500 nucleotide length of the ACTOL transcript). Thus, cloning the *Drosophila* ADH gene behind a yeast polymerase II promoter is not sufficient to allow splicing to occur.

Figure 3 shows the result of S1 nuclease mapping using a

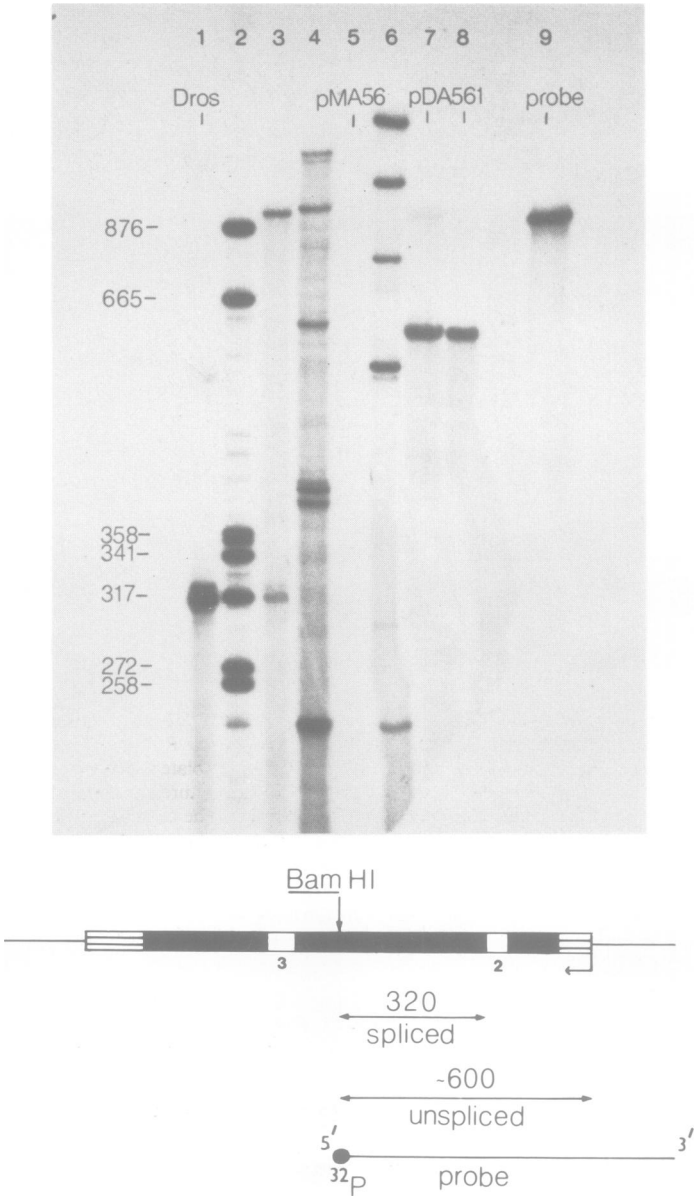

Fig. 3. S1 nuclease assay for splicing of *Drosophila ADH* intron 2. The probe was a 930-bp *Bam*HI-*Hpa*I fragment of the *Drosophila* ADH gene ^{32}P-labelled at the 5′ terminus of the *Bam*HI site. The assay was performed as described for Figure 2. The diagram indicates the origin of the probe and the protected fragments. **Lanes 2** and **4**, DNA size markers; **1** and **3**, *Drosophila* poly(A)$^+$ RNA (two different preparations); **5**, **7** and **8**, RNA from *S. cerevisiae* strain 302-21 transformed with pMA56 **(5)** or pDA561 **(7** and **8)**; **lane 9**, undigested probe.

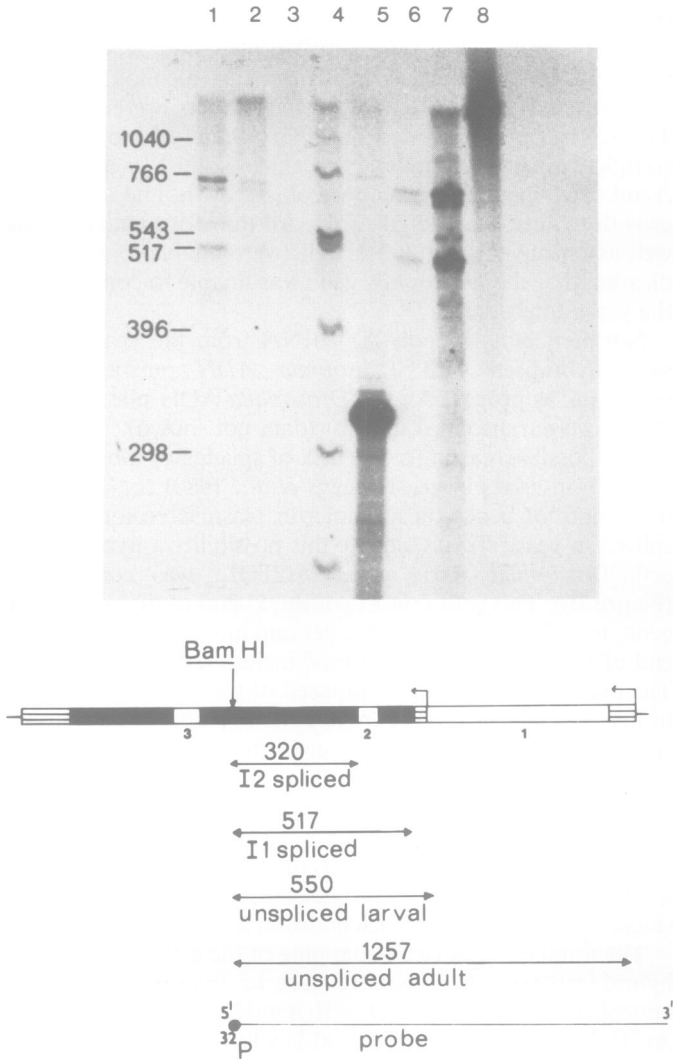

Fig. 4. S1 nuclease assay for splicing of *Drosophila ADH* intron 1. The diagram shows on the top line the *Drosophila* ADH gene (symbols in Figure 1A), and the bottom line shows the origin of the probe which was ^{32}P-labelled at the 5′ terminus at the *Bam*HI site of the *Drosophila* ADH gene and extended upstream beyond the adult transcription initiation site. The lengths are indicated for protected probe which could be expected depending upon whether intron 2 (I2) was spliced, or whether only intron 1 (I1) was spliced, or whether transcription was initiated at the larval or adult promoters and transcripts remained unspliced. S1 nuclease digestion was carried out at a concentration of 300 units S1 nuclease/ml and protected fragments were detected by polyacrylamide (5%) gel electrophoresis and autoradiography. RNA from *S. cerevisiae* transformed by: **1**, pMA567; **2**, pMA565; **3**, pMA56; **6**, pMW57; **7**, pMW55; **5**, poly(A)$^+$ from *Drosophila;* **8**, undigested probe; **4**, DNA size markers.

930-bp *Hpa*I-*Bam*HI restriction fragment 5′ end-labelled at the *Bam*HI site to detect splicing of intron 2. Correctly spliced transcripts should protect a fragment of 320 nucleotides (lane 1), however RNA from cells transformed with pDA561 protected ~600 nucleotides (lanes 7 and 8). This shows that splicing of intron 2 does not occur and the size of the protected fragment is consistent with transcription initiating at the yeast promoter. The absence of splicing of introns 2 and 3 and the proposed positions of the 5′ and 3′ ends account for the sizes of transcripts observed on Northern blots with pDA561 and pDA563 (1700 and 2100 nucleotides, respectively).

The results of an analysis of splicing of intron 1 are shown in Figure 4. A 1.3-kb *Sal*I-*Bam*I DNA fragment 5′ end-labelled at the *Bam*HI site was used. Correct splicing of intron 1 should give protection of 517 nucleotides of the probe, while unspliced larval and adult transcripts should protect 550 and 1257 nucleotides, respectively. A complex series of protected fragments is observed which is the same for RNA from cells transformed with plasmids containing either of the larger inserts (*Eco*RI-*Eco*RI or *Sal*I-*Eco*RI restriction fragments) with (lanes 1 and 2) or without (lanes 6 and 7) the yeast *ADC* promoter. The sizes of the major protected fragments are 505, 517, 550, 660–680 and 700–720 nucleotides.

These protected fragments could represent the positions of splice acceptor sites or 5′ ends of transcripts, however, the

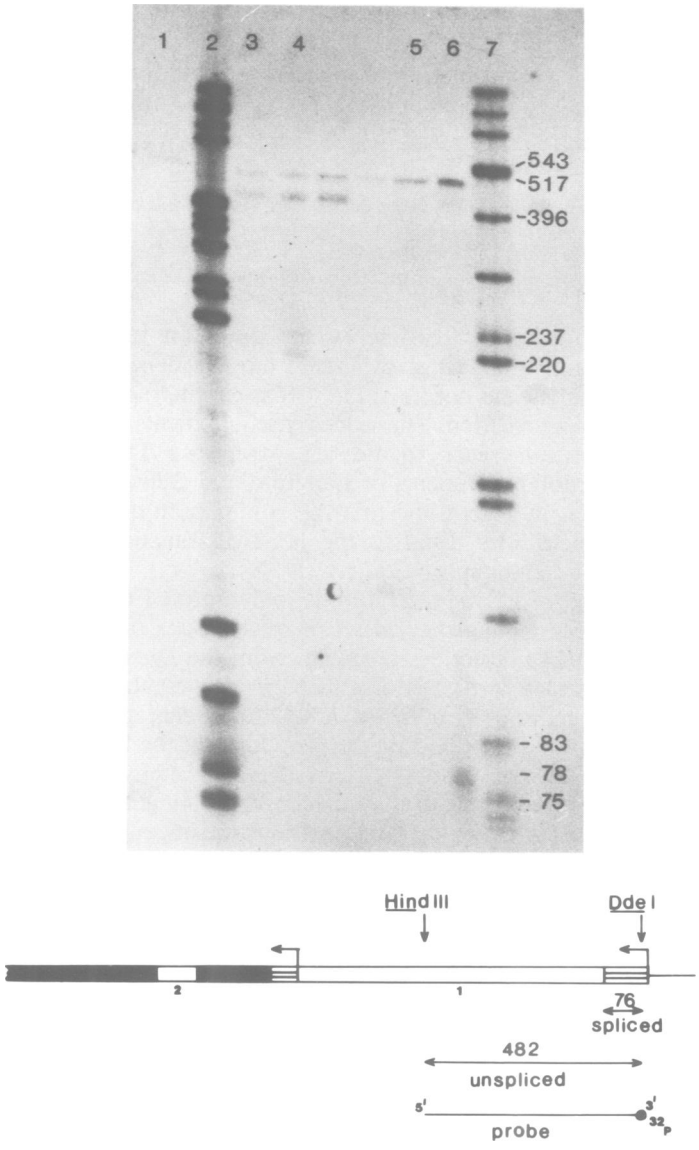

Fig. 5. S1 nuclease analysis for splicing of *Drosophila ADH* intron 1. The probe was a 484-bp *Dde*I-*Hind*III fragment of the *Drosophila* ADH gene ^{32}P-labelled at the 3′ terminus of the *Dde*I site. The assay was performed as described for Figure 4. Protected fragments were detected by polyacrylamide (8%) gel electrophoresis and autoradiography. **Lane 1**, probe; **lanes 2** and **7**, size markers; **lanes 3–5**, RNA from *S. cerevisiae* 302-21 transformed with pDA565 **(lane 3)**, pMW55 **(lane 4)**, or pMA56 **(lane 5)**. **Lane 6**, poly(A)$^+$ RNA from *Drosophila*.

517 nucleotide fragment is consistent with correct splicing, while the less abundant 550 nucleotide fragment coincides with the location of the 5′ ends of larval *ADH* transcripts. The origin of the other major protected fragments is not obvious, however, the 700–720 nucleotide fragment terminates ~70–90 nucleotides downstream of a TATA-like sequence, and may therefore represent the 5′ end of a transcript. (In yeast genes TATA sequences are positioned between 40 and 180 nucleotides upstream from the transcription start point; Sentenac and Hall, 1982).

The results of S1 nuclease mapping from the 5′ end of the gene using a 484-bp *Dde*I-*Hind*III fragment 3′ end-labelled at the *Dde*I site, are shown in Figure 5. Correct splicing of intron 1 results in protection of a fragment of 76 nucleotides (lane 6), while RNA from yeast cells transformed with pDA565 or pMW55 protects a fragment of 420 nucleotides

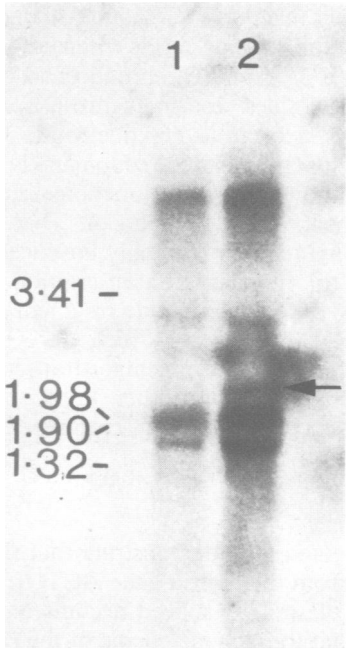

Fig. 6. Northern analysis of DA565 transcripts in an *rna2* mutant at 24°C and 37°C. pDA565 was introduced into the yeast strain RY101 by crossing it with 302-21 containing pDA565. A *TRP*$^+$ *rna2*$^-$ isolate was grown at 24°C overnight then split into two; one half of the culture remained at 24°C and the other was incubated at 37°C for 1 h. The cells were then treated with sodium azide as described by Hereford *et al.* (1981) and total RNA prepared as described in Materials and methods. **Lane 1**, 24°C; **lane 2**, 37°C for 1 h. The sizes shown are those of λDNA digested with *Hind*III + *Eco*RI. The arrowhead indicates the position of the additional band in **lane 2**. Bands higher up are due to the presence of DNA in the unfractionated RNA preparations.

(plus minor fragments of 231 and 224 nucleotides with pMW55) as well as full length probe. The end of the 420 nucleotide protected fragment maps to the sequence GCAGTAATT which resembles a donor splice junction sequence.

A temperature-sensitive conditional lethal mutation ts368 in the *RNA2* gene of *S. cerevisiae* causes the accumulation, after a brief incubation at the non-permissive temperature, of high mol. wt. precursor transcripts of genes containing introns (Rosbash *et al.*, 1981; Teem and Rosbash, 1983), and it has been suggested that this mutant is defective in some aspect of RNA splicing. To determine whether *RNA2*-dependent processing of the *Drosophila ADH* transcripts occurred, RNA was isolated from a pDA565 transformant of *S. cerevisiae* strain RY101 carrying the ts368 mutation and analysed by Northern blotting (Figure 6). RNA from RY101 grown at 24°C (permissive temperature) gave two bands corresponding to *ADH* transcripts of ~1600 and 2000–2100 nucleotides. RNA isolated after growth for 1 h at 37°C (non-permissive temperature) gave an additional band at 2400 nucleotides which corresponds to the length expected for an unspliced precursor RNA initiated at the *ADC* promoter. This result suggests that the 2400 nucleotide transcript is a precursor of *RNA2*-dependent processing.

The results of the S1 mapping experiments taken in conjunction with the evidence for *RNA2*-dependent processing, indicate that one or more splicing reactions occur in the leader sequence of *ADH* transcripts. For example, 2400 nucleotide primary transcripts of pDA565 are probably processed in an

*RNA*2-dependent reaction such that a 520 nucleotide 5′ exon (represented by the 420 nucleotide fragment in Figure 5, lane 3) is spliced to a 3′ exon. The broad band of transcripts of 2000−2100 nucleotides seen in Figure 6 is compatible with splicing of the 5′ exon to a 1600 nucleotide 3′ exon with the same 3′ splice junction as in *Drosophila* RNA, and possibly with other additional 3′ splice junctions represented by the multiple fragments seen in Figure 4. The 1600 nucleotide transcripts in Figure 6 are probably unspliced transcripts initiated close to the larval transcription initiation site (represented by the 550 nucleotide fragment in Figure 4) but may include RNA fragments which were the products of endonucleolytic cleavage of precursor transcripts without being subsequently ligated together in a splicing reaction. Definitive evidence for splicing requires a more detailed analysis.

Discussion

Results presented here demonstrate that the actin portion of transcripts from the hybrid gene ACTOL encoded on a 2 micron DNA plasmid is spliced accurately and efficiently. This suggests that the lack of splicing of the *Drosophila ADH* intron 3 from this same hybrid transcript is not due to a general inability of the transcripts of plasmid-encoded genes to be spliced, nor to incorrect initiation of transcription (as was observed in the rabbit β-globin system, Beggs *et al.*, 1980) nor to incorrect transcription termination, but that some property of the *Drosophila ADH* sequence is incompatible with the yeast splicing mechanism. The lack of splicing of this intron could be due to a general inability of the cells to splice more than one intron from a transcript. However, Langford *et al.* (1983) have shown, using a hybrid yeast actin-RP51 gene, that two introns can be spliced from the same transcript, and Miller and Nasmyth (personal communication) have recently found that the yeast *MAT*a gene contains two introns. Langford and Gallwitz (1983) also found that the transcripts of a duck α-globin and an *Acanthamoeba* actin gene encoded on an autonomously replicating *(ars)* vector were not spliced in yeast. The lack of splicing of heterologous gene transcripts in yeast is therefore likely to be a general phenomenon, with the *Drosophila ADH* intron 1 being unusual.

The seven introns of yeast protein-coding genes whose sequence is known share a seven base homology, $^{G}/_{T}GTA^{T}/_{C}GT$ at the 5′ end (Langford and Gallwitz, 1983; Pikielny *et al.*, 1983), and deletion studies of this region indicate that the G at position 6 (nucleotide 5 of the intron) is important (Pikielny *et al.*, 1983; Rosbash, personal communication). Figure 7 shows the sequences at the exon-intron boundaries of the *Drosophila* ADH gene. All three introns have donor splice junctions resembling, but not identical to, the yeast consensus sequence, however we found no evidence for the use of any of these donor splice junctions in yeast. The sequence AGTAATT is probably the site of the 5′ splice junction in yeast. Presumably in yeast some additional feature of the transcript is required to determine the position of donor splice sites.

In addition, all the yeast introns so far sequenced contain a conserved sequence TACTAAC (ATTTACTAAC if a mismatch of up to four bases is permitted) positioned 20−60 nucleotides upstream from the 3′ splice site (Langford and Gallwitz, 1983; Pikielny *et al.*, 1983). Deletion analyses and intron reconstruction experiments indicate that this internal

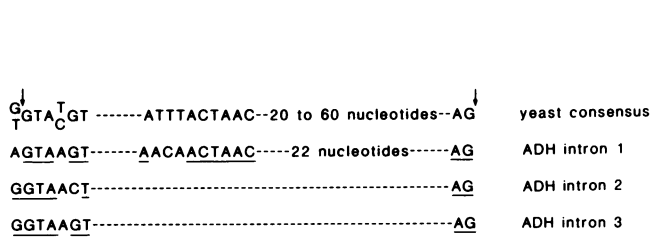

Fig. 7. Sequences around the splice junctions of the *Drosophila* ADH gene (Benyajati *et al.*, 1983) and the proposed consensus sequences for yeast introns (Langford and Gallwitz, 1983; Pikielny *et al.*, 1983). The arrows indicate the positions of splice points. Homologous sequences are underlined.

conserved sequence (ICS) serves an important function in splicing, and Pikielny *et al.* (1983) have evidence that an endonucleolytic cut occurs at the ICS as an intermediate step in the splicing reaction. The sequence requirements for the 3′ splice junction seem to be less stringent. The intron reconstruction experiments of Langford and Gallwitz (1983) suggest that in general the first AG a minimum distance of 10−15 nucleotides distal to the ICS will function as the preferred acceptor splice site.

Of the *Drosophila ADH* introns, only intron 1 contains a sequence resembling the yeast ICS (seven bases out of 10), positioned 24 nucleotides upstream from the *Drosophila* 3′ splice site. Data presented in this paper suggest that this 3′ splice junction is used in an *RNA*2-dependent processing reaction in yeast, supporting the proposal that the ICS determines the position of the 3′ splice site, and thereby fulfilling one of the predictions of the model of Pikielny *et al.* (1983).

The process of splicing of nuclear protein-coding transcripts in all eukaryotes has presumably arisen from a common origin, however, the inability of *S. cerevisiae* to accurately splice the introns from heterologous transcripts reflects the evolutionary divergence of the splicing mechanism in this yeast compared with other eukaryotes. We present here the first example of the splicing of a heterologous transcript in yeast. The presence of an ICS-like sequence close to the 3′ splice site of this intron supports the proposal that this is an important factor in determining the acceptor splice site. Our results suggest, however, that the choice of the donor splice junction requires some, as yet undetermined, feature in the RNA in addition to the 5′ splice consensus sequence, although the deletion analyses of Gallwitz (1982) suggest that the 5′ half of the actin intron up to 18 nucleotides from the donor splice site may be deleted without any serious effect on splicing. Some of the transcripts observed in this work may represent unligated splicing intermediates and this may reflect inefficient ligation of the products of endonucleolytic cleavage in the absence of a precise consensus sequence at the donor splice site. Experiments now in progress in several laboratories to reconstruct genes with introns using the various sequence components now known to be important for splicing will hopefully result in a detailed understanding of the sequence requirements for splicing polymerase II transcripts in *S. cerevisiae*.

Materials and methods

Bacterial strains, yeast strains and plasmids

Escherichia coli strains DH1 (Hanahan, 1983) or HB101 (Maniatis *et al.*, 1982) were used for the preparation of plasmid DNA. *S. cerevisiae* strain 302-21 (α *adc*1 *adm adr*2 *trp*1 *his*4; Williamson *et al.*, 1980) was used unless otherwise stated. The *rna*2 strain of *S. cerevisiae* was RY101 (*a rna*2-1 *ura*3-1 *trp*1-289 *his*3-532 *ade*2-10; R.Young, Stanford).

Plasmid vectors pMA56 (Valenzuela *et al.*, 1982) and pMW5 were from B.Hall. pMA56 contains the promoter from the yeast *ADC* gene, the origin of

replication from 2 micron DNA, the *TRP*1 gene of yeast, and pBR322 sequences. The related vector pMW5 has the yeast *ADC* promoter fragment replaced by the *Eco*RI-*Bam*HI fragment from the region of the *Tet*^{r} gene of pBR322. The *D. melanogaster* ADH gene was subcloned from ps*AC*1 (Goldberg, 1980), and the yeast actin gene was from pYA208 (Gallwitz and Sures, 1980). Other plasmids were constructed as described in the figure legends.

Transformation procedures were essentially as described previously (Scott *et al.*, 1983; Beggs, 1978).

Enzymes

S1 nuclease was obained from Sigma, AMV reverse transcriptase was from Life Sciences Inc., FL, calf intestinal phosphatase and polynucleotide kinase were from Boehringer Mannheim. Restriction endonucleases were obtained from New England Biolabs or Bethesda Research Laboratories. T4 DNA ligase was a gift from J.Wolfe. Radiochemicals were purchased from Amersham International.

Preparation and analysis of RNA

Total RNA from yeast was prepared using a modification of the method described by Beggs *et al.* (1980), in which heparin and sucrose were omitted from the two extraction buffers. Poly(A)$^+$ and poly(A)$^-$ RNA species were separated using oligo(dT)-cellulose chromatography (Aviv and Leder, 1972).

Drosophila poly(A)$^+$ RNA was obtained from 3- to 5-day old flies (strain Oregon R) by the method of Kidd and Glover (1981).

In the 'Northern blotting' procedure, RNA was denatured by treatment with glyoxal followed by electrophoresis through agarose (McMaster and Carmichael, 1977) and transfer to Gene Screen (New England Nuclear).

S1 nuclease digestions were carried out using poly(A)$^+$ RNA by the method of Berk and Sharp (1977) as modified by Weaver and Weissmann (1979). DNA probes were end-labelled as described by Maxam and Gilbert (1980) or Maniatis *et al.* (1982) or uniformly labelled by nick-translation (Rigby *et al.*, 1977). S1 digestion products were analysed by electrophoresis in 3%, 5% or 8% polyacrylamide gels containing 7 M urea (Sanger and Coulson, 1978). Autoradiography was carried out at −70°C using Fuji RX film and Mach 2 fast tungstate intensifying screens.

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