Altered Growth and Cell Walls in a Fucose-Deficient Mutant of Arabidopsis

Wolf-Dieter Reiter, Clint C. S. Chapple, Chris R. Somerville

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rates, temperatures, and conditions, of a battery with a solid sulfur cathode, a polysulfide interface, and an aluminum anode. Typical open circuit voltages are 1.28 to 1.30 V. Under moderate to high rate conditions [1-ohm load over a "D" cell configuration (*24*)], the discharge time of 15 to 18 hours (Fig. 3) is over twice the 6.5-hour discharge obtainable in conventional alkaline batteries (6) and an increase of 30% compared with the previously described aluminum-polysulfide cell (*9*). The measured specific energy capacity of the Al-S battery (1-ohm discharge) is 220 W·hour/kg on the basis of active materials. Highly concentrated anolytes induce cathodic polarization losses (Fig. 3), a phenomenon that one can minimize by increasing cell temperature, increasing the ionic strength, or substituting disulfide for tetrasulfide in the catholyte interface.

The measured specific energy of 220 W·hour/kg of this cell can only provide an approximate comparison with the capacities of aqueous batteries in a more mature state of development. Conventional aqueous batteries typically achieve an experimental specific energy of 10 to 25% of the theoretical. The mechanically rechargeable Zn-air battery is considered to have a high measured specific energy of up to 110 W·hour/kg, and alkaline batteries with low discharge rates ($Zn-MnO_2$) have a specific energy of up to 95 W·hour/kg (*2*, *5*).

A further increase in Al-S power and specific energy may be accessible with a recently described Al-redox, mechanically rechargeable flow cell configuration with a high power density in which solvent may be recycled while electrolyte flows into and through the cell (*25*). The solid sulfur cathodes can support the requisite current densities for this configuration (Fig. 2, inset). This configuration, as modeled with an Al-ferricyanide battery, took advantage of the high currents sustainable for the reduction of ferricyanide (up to 0.5 A/cm^2 on planar electrocatalysts, and in excess of 2 A/cm^2 on porous electrocatalysts) and permitted better utilization of anode materials (*25*). Replacement of ferricyanide with a solid sulfur cathode may considerably enhance the energy capacity of this configuration.

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10. From Eq. 2, 2 faradays of electrons are present for each mole of S and H_2O (0.032 + 0.018 kg). We can get the appropriate charge units per kilogram using standard conversions: 1 faraday = 96,500 C, 3600 C = 1 A·hour.
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Altered Growth and Cell Walls in a Fucose-Deficient Mutant of *Arabidopsis*

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A biochemical screening procedure was developed to identify mutants of *Arabidopsis thaliana* in which the polysaccharide composition of the cell wall was altered. Over 5000 ethyl methanesulfonate–mutagenized plants were analyzed by this method, leading to the identification of 38 mutant lines. One complementation group of mutants was completely deficient in L-fucose, a constituent of pectic and hemicellulosic polysaccharides. These mutant plants were dwarfed in growth habit, and their cell walls were considerably more fragile than normal.

The primary cell wall of higher plants determines cell shape and size during plant growth and development. Cell walls also provide mechanical support for plant tissues and organs and are intimately involved in a multitude of biological processes, such as cell-cell recognition and interaction, defense responses, and tropic responses (*1*). Plant cell walls are primarily composed of the polysaccharide components cellulose, hemicelluloses, and pectins (*2*). Cellulose microfibrils cross-linked by xyloglucan molecules are believed to serve as major load-bearing elements within the wall; however, the precise functions of the noncellulosic cell wall polysaccharides are poorly understood. To elucidate the roles of individual cell wall polysaccharides and to clone genes involved in their synthesis, we have taken a genetic approach by screening mutagenized *Arabidopsis* plants for alterations in their polysaccharide composition. One particularly informative class of mutant lines lacked fucose in their cell wall polysaccharides. Plants in this class had changes in their growth habit and in the mechanical properties of their walls.

From an ethyl methanesulfonate–mutagenized population of *Arabidopsis* plants (*3*), 5200 were screened for alterations in the monosaccharide composition of cell wall polysaccharides in leaves. For this purpose, acid hydrolysates of cell walls were analyzed by gas chromatography of alditol acetates (*4*). This screening strategy was based on the concept that many

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genetic changes in cell wall polysaccharide composition would be detectable as alterations in the relative amounts of the constituent monosaccharides. After several rounds of screening and selection (*5*), we obtained 38 mutant lines that showed heritable changes in cell wall composition. Most of these lines showed substantial changes in the relative amounts of one or more cell wall–derived monosaccharides, but not below 50% of wild-type amounts of any of the sugar monomers. However, five lines were almost completely devoid of L-fucose in shoot-derived cell wall material. This monosaccharide is a constituent of pectic and hemicellulosic cell wall polysaccharides (*2*) and is present in some glycoproteins (*6*). In *Arabidopsis*, L-fucose accounts for ~0.5% of the dry weight of cell wall material (*7*).

F_1 progeny obtained from crosses between the five fucose-deficient lines had the same low fucose content as the parental lines, indicating that they carried allelic mutations at a locus that we have designated *mur1*. In crosses to wild-type plants, the fucose deficiency segregated essentially 3:1 (*8*), indicating that it represented a single recessive Mendelian trait. After four backcrosses to wild-type plants (*9*), two mutant lines carrying the independently derived alleles *mur1-1* and *mur1-2* were chosen for further study.

Both *mur1* lines typically contained less than 2% of the wild-type amount of fucose in aerial parts of the plant body (*10*); however, the fucose content in total root polysaccharides was only reduced by about 40% (Fig. 1). Mutant plants grown axenically in the presence of 10 mM L-fucose contained essentially wild-type amounts of fucose in both root- and leaf-derived cell wall material (Fig. 1), suggesting that the mutant phenotype was caused by an inability to synthesize L-fucose in the shoot (*11*) and a reduced ability to synthesize L-fucose in the root, although more complex explanations cannot be ruled out.

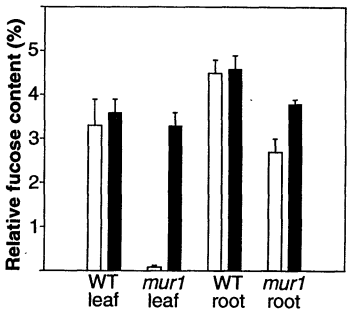

Fig. 1. Relative fucose content of leaves and roots from wild-type (WT) and *mur1* plants grown in the presence (filled bars) or absence (open bars) of L-fucose. The fucose content is given as the percentage of total neutral cell wall monosaccharides (excluding glucose). Error bars are standard deviations with a sample size of six. Plants were grown under continuous fluorescent light (70 to 100 $\mu E\ m^{-2}\ s^{-1}$) at 23°C on MS plates (*28*) with or without 10 mM L-fucose. Leaves and roots were harvested from individual plants in late rosette stages and analyzed for the monosaccharide composition of their walls with the use of the standard screening procedure (*4*), except that five rather than two ethanol extractions were performed.

Mutant plants grown in pots under continuous light conditions were distinguished from wild-type plants by a dwarfed growth habit characterized by shorter petioles, shorter internodes, decreased height, and reduced apical dominance (Fig. 2). The degree of dwarfing was variable within mutant populations, and extremely stunted plants were occasionally observed. This phenotypic variability may be due to some nonuniformity in the microenvironment of individual plants such as differences in soil conditions. All of the morphological phenotypes of the mutant plants were found in both independently derived *mur1-1* and *mur1-2* lines, indicating that both fucose deficiency and altered morphology were caused by the *mur1* mutation (*12*). Mutant plants grown axenically in the presence of L-fucose were phenotypically indistinguishable from wild-type plants (Fig. 3), confirming that the lack of fucose caused the alterations in shoot growth.

Fig. 2. Growth habits of wild-type and *mur1* plants. Wild-type plant (**A**), and *mur1* plants showing moderate (**B**) and extremely dwarfed (**C**) growth habits. Plants were grown at 23°C under continuous light (70 to 100 $\mu E\ m^{-2}\ s^{-1}$) in pots as described (*26*).

The dwarfed appearance of the *mur1* plants closely resembled the morphology of mutants affected in the synthesis or perception of the growth regulators auxin (*13*) and gibberellin (*14*). When sprayed with gibberellin A_3 or the synthetic auxin 2,4-dichlorophenoxy acetic acid, *mur1* plants persisted in the dwarfed growth habit, suggesting that the alterations in morphology were not caused by deficiencies of these phytohormones. Furthermore, the *cgl* mutant of *Arabidopsis*, which lacks fucose in glycoproteins because of its inability to process N-linked glycans (*15*), is morphologically indistinguishable from wild-type plants, suggesting that the abnormal growth habit of the *mur1* plants is not caused by changes in glycoprotein fucosylation.

During routine handling of the *mur1* plants we noted that the elongating parts of the inflorescences were quite fragile. Load-extension curves of such segments (*16*) indicated that the force required to tear segments of comparable diameter was more than twofold reduced in mutant plants in comparison to wild-type; the energy required for breakage was reduced by a factor of 5 (Fig. 4A). Scanning elec-

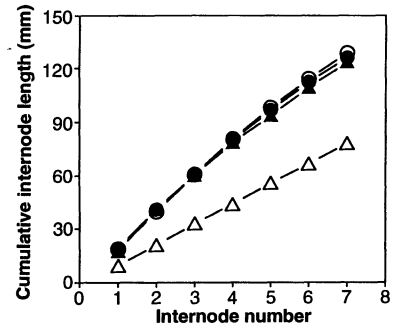

Fig. 3. Cumulative internode lengths of wild-type plants (circles) and *mur1* plants (triangles) grown in the presence (filled symbols) or absence (open symbols) of L-fucose. Plants were initially grown at 23°C on 19.2 mesh nylon nets on the surface of MS plates (*28*) with or without 10 mM L-fucose. Fifteen days after planting, the plates were placed at 10°C for 12 days, then the nylon nets carrying the plants were transferred to 10-liter flasks containing 50 g of perlite and 2 liters of 0.5× concentrated nutrient solution (*26*) with or without 5 mM L-fucose. Nylon nets (19.2 mesh) supported by air-filled polypropylene tubes were used as rafts to provide buoyancy. Plants were grown for 8 weeks at 10°C with continuous air supply and illumination. Cumulative internode lengths in the nongrowing basal parts of the plants were determined by measurement of the lengths between the first (that is, oldest) silique on the main inflorescence and each of the seven subsequent siliques. All plants were evaluated (at least 24 plants per flask) and averages were calculated. The experiment was repeated once with the same results.

tron microscopy of the broken ends indicated rupture exclusively within the walls; cell separation at the middle lamella was not observed (Fig. 5). The overall anatomy of the segments was not obviously altered, and the determination of wall thickness by quantitation of cellulose (*17*) and total cell wall–derived monosaccharides (*18*) did not reveal significant differences between wild-type and mutant plants, indicating that the *mur1* mutation did not reduce the amounts of polysaccharides in the cell walls but affected mechanical properties of the primary wall. When mutant plants were supplied with exogenous L-fucose, the apical regions of their inflorescences displayed the mechanical strength typical for wild-type plants (Fig. 4B). The wall strength of the *cgl* mutant (N-linked glycan processing defect) (*15*) was not significantly different from that of wild-type plants (Fig. 4A), indicating that the weakened wall structure of the *mur1* plants was not an indirect consequence of altered protein fucosylation.

In dicotyledonous plants like *Arabidopsis*, the most common fucose-containing cell wall polymers are rhamnogalacturonan II, a pectic component of unknown function (*19*), and xyloglucan, a hemicellulose believed to coat and cross-link cellulose microfibrils (*20*). Xyloglucan seems to regulate extension growth on account of its susceptibility to endoglucanases (*21*) and endotransglycosylases (*22*) within the wall. Such specific degradation of xyloglucan may lead to wall-loosening events during extension growth. On the basis of energy calculations on xyloglucan conformers, the fucose-containing side chain has been proposed to stabilize conformations that can efficiently bind to cellulose (*23*). In the *mur1* mutants, alterations in xyloglucan cleavability or in its interactions with cellulose may form the basis of the decreased wall strength; however, the exact mechanism remains to be established.

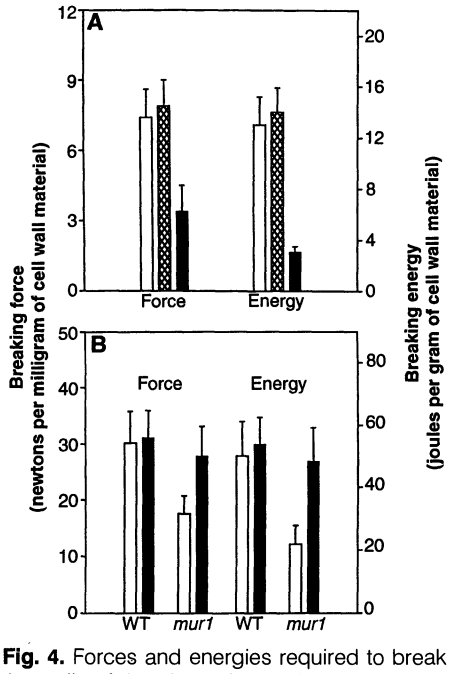

Fig. 4. Forces and energies required to break the walls of the elongating regions of inflorescence stems. Error bars are standard deviations with a sample size of 16 (A) or 25 (B). (**A**) Breaking forces and energies measured for wild-type (open bars), *cgl* (cross-hatched bars), and *mur1* plants (filled bars). Plants were grown in pots as in Fig. 2. (**B**) Breaking forces and energies measured for wild-type (WT) and *mur1* plants grown axenically in the presence (filled bars) or absence (open bars) of L-fucose. Axenic growth conditions were essentially as in Fig. 3, except that the perlite was omitted and the growth temperature was continuously 23°C.

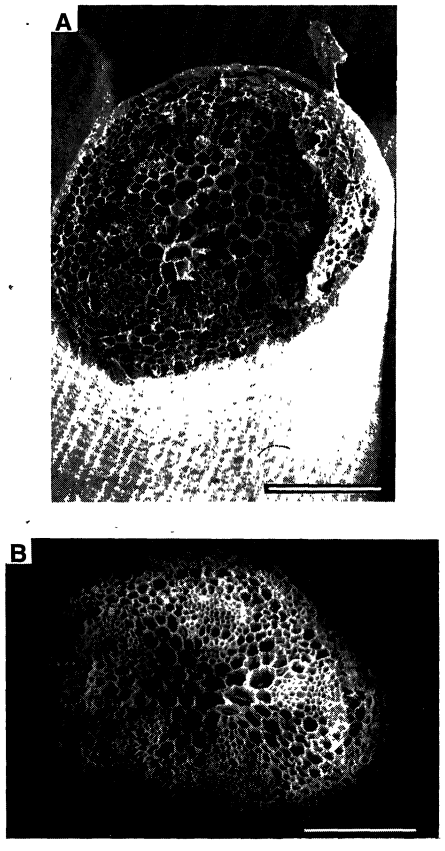

Fig. 5. Scanning electron micrographs of the surface of broken inflorescence stems. (**A**) Wild-type plants. (**B**) *mur1* plants. Scale bars, 100 μm. Inflorescence stems from plants in middle flowering stages were cut about 7 cm from the apex, held at both ends, and manually extended until broken. Breakage typically occurred within the growing region between 0.5 cm and 2 cm from the apex. Samples were fixed for 1 to 2 hours in 4% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.4. After dehydration in a graded ethanol series, the plant material was critical point dried with CO_2 as a transitional fluid, coated with gold (20 nm thickness), and examined in a JEOL JSM-35CF scanning electron microscope (Japan Electron Optics Ltd.).

The turnover of xyloglucan during extension growth is believed to lead to the formation of a fucose-containing "oligosaccharin" fragment (XG9) that inhibits its auxin-induced elongation growth at nanomolar concentrations, thereby establishing a feedback loop to prevent excessive cell wall extension (*24*). The anti-auxin activity of XG9 is dependent on the presence of the fucose residue (*25*). Considering that the fucose-deficient *mur1* plants show a dwarfed growth habit presumably caused by a reduction in extension growth, we believe that the oligosaccharin hypothesis needs to be reexamined, because in its current form this hypothesis would predict excessive rather than reduced extension growth in the *mur1* plants as a result of the probable absence or reduction of the auxin antagonist. Although the pleiotropic effects of the *mur1* mutation interfere to some extent with an evaluation of the oligosaccharin hypothesis, our data do not support a pivotal role of this proposed feedback loop for plant development.

In summary, the information gained by characterization of the fucose-deficient mutants illustrates the feasibility and utility of a genetic approach to the study of the synthesis, structure, and function of plant cell walls.

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3. Mutagenesis of *Arabidopsis* seeds was as described (*26*).
4. Mutagenized plants were grown under continuous fluorescent light (70 to 100 $\mu E\ m^{-2}\ s^{-1}$) at 23°C in pots as described (*26*). Single leaves were harvested from 3- to 4-week-old seedlings and extracted twice for 1 hour each at 70°C with 1 ml of 70% ethanol and once for 5 min at room temperature with 1 ml of acetone. The extracted leaves were dried under vacuum and hydrolyzed in 1 M H_2SO_4 for 1 hour at 121°C. The released monosaccharides were converted into alditol acetates as described (*18*) and quantified by gas chromatography in splitless mode on a 30-m SP2330 column (Supelco; 0.75-mm inner diameter) using helium at a flow rate of 5 ml/min. The temperature program was as follows: 2 min at 160°C, 20°C per minute gradient to 200°C, held at 200°C for 5 min, 20°C per minute gradient to 245°C, held at 245°C for 12 min. Relative amounts were calculated for the cell wall–derived sugars rhamnose, fucose, arabinose, xylose, mannose, and galactose, and these values were used to construct histograms encompassing at least 100 plants. Plants were scored as putative mutants if the relative amount of at least one monosaccharide was outside of its standard distribution.
5. The initial screen yielded 166 putative mutants, 70

of which were confirmed in the next generation. All 70 lines were backcrossed to wild-type plants, and at least 60 F_2 plants obtained by selfing of the F_1 progeny were scored for reselectability of the mutant phenotype to eliminate lines where the altered cell wall composition was caused by the combined action of several mutations.
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7. Purified cell wall material from *Arabidopsis* leaves was evenly suspended at a concentration of 2 mg/ml in 1 M H_2SO_4 containing *myo*-inositol (200 μg/ml) as an internal standard. This suspension was divided into six identical portions of 250 μl each, and three of these samples were spiked by addition of 5 μg of L-fucose each. The fucose content of the walls was calculated after gas chromatographic separation of alditol acetates (*4*), using the spiked samples as a reference. The amount of cell wall–derived fucose in the samples was 2.33 ± 0.25 μg.
8. Out of 167 F_2 plants, 128 were phenotypically wild type and 39 phenotypically mutant; $\chi^2 = 0.17$, $P > 0.6$.
9. Backcrossing to wild-type plants followed by the reselection of the mutant phenotype from segregating populations serves to reduce the number of background mutations.
10. The amount of residual fucose in leaves from pot-grown *mur1-2* plants was 1.0 ± 0.6% of the wild-type amount (sample size of 10); plants grown axenically on plates showed an approximately fourfold higher amount of residual fucose.
11. Gas chromatographic quantitation of L-fucose released from the sugar nucleotide fraction of leaf material indicated approximately 25-fold less L-fucose in the mutant than in the wild type, corroborating the conclusion that the *mur1* mutation affects the de novo synthesis of L-fucose.
12. Because the *mur1-1* and *mur1-2* lines were derived from independent mutagenesis events, they should differ in the location of background mutations, essentially eliminating the possibility that the visible phenotypes observed in both mutant lines were due to a mutation distinct from the *mur1* locus.
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16. Inflorescences were harvested from plants in their middle flowering stages and prepared for measurements of tensile properties as follows: The whole apex of the inflorescence including the oldest mature flower was fixed between two layers of adhesive tape, then the lower part of the inflorescence was similarly taped, leaving a 4.5-mm segment of free stem between attachments. Flowers within this region were removed with fine scissors, and the tapes were cut to final sizes of about 3 by 3 mm each. Care was taken to avoid mechanical stress or damage to the stem segments during these steps. The taped segments were immediately frozen in liquid nitrogen and stored at −80°C. Breaking force measurements were carried out with the use of a custom-built extensiometer as described by R. Prat and G. Paresys [*Plant Physiol. Biochem.* **27**, 955 (1989)]. The taped segments were thawed at room temperature for 2 min and inserted between the clamps of the extensiometer (distance between clamps, 5.6 mm). The force exerted by the clamps was on the taped regions of the stems only, avoiding damage to the non-taped portion of the segments. The clamped segments were submerged in deionized water and extended at a rate of 2 mm/min until broken. Breakage occurred at the ends or in central parts of the segments with similar frequencies. A load-extension curve was recorded and evaluated for the force and energy required to break each segment. Breaking forces were usually in the 0.1 to 0.15 N range for segments from *mur1* plants and 0.25 to 0.35 N for segments from wild-type plants. To correct for differences in wall areas between individual plants, we cut the broken segments at their taped ends and quantified the amount of cell wall material including cellulose (*27*) by gas chromatography of alditol acetates as described (*4*). The sum of the amounts of the cell wall–derived monosaccharides arabinose, xylose, galactose, and glucose were used for this normalization procedure.
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Group II Intron RNA Catalysis of Progressive Nucleotide Insertion: A Model for RNA Editing

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The self-splicing *bI1* intron lariat from mitochondria of *Saccharomyces cerevisiae* catalyzed the insertion of nucleotidyl monomers derived from the 3′ end of a donor RNA into an acceptor RNA in a 3′ to 5′ direction in vitro. In this catalyzed reaction, the site specificity provided by intermolecular base pair interactions, the formation of chimeric intermediates, the polarity of the nucleotidyl insertion, and its reversibility all resemble such properties in previously proposed models of RNA editing in kinetoplastid mitochondria. These results suggest that RNA editing occurs by way of a concerted, two-step transesterification mechanism and that RNA splicing and RNA editing might be prebiotically related mechanisms; possibly, both evolved from a primordial demand for self-replication.

The discovery of RNA molecules with enzymatic activities (ribozymes) and the diversity of the reactions that they catalyze have provoked interest in theories that suggest that early replicating systems were probably made of RNA or an RNA-like derivative (*1–3*). Zaug and Cech (*4*) demonstrated that RNA polymerization in a classical 5′ to 3′ polarity could be catalyzed by the self-splicing group I *Tetrahymena* intron. A pentamer of cytidylic acid (C_5) is converted to cytidylic acids up to C_{30} by cleavage-ligation reactions (transesterification). The specificity for polycytidylic acids relies on base pair interactions with the intron internal guide sequence (IGS). A parallel between the function of the IGS sequence in RNA splicing and the proposed role for the guide RNAs (gRNAs) in RNA editing in kinetoplastid mitochondria (*5–11*) has been recognized (*12, 13*).

Theoretical considerations (*13*) and experimental evidence (*12*) suggest that post-transcriptional uridine insertion and deletion occur by a series of splicing-like transesterification reactions (*12, 13*). The postulated chimeric intermediate with the gRNA covalently joined to the 3′ portion of the mRNA (*12*) has been identified in vitro (*14, 15*). Although formation of the gRNA-mRNA chimeric intermediate can also be explained by separate cleavage and ligation reactions (*16*), other findings (*14, 15*) support the concerted transesterification model for uridine insertion in a 3′ to 5′ direction (*12, 13*) and suggest an evolutionary analogy to the catalytic mechanisms involved in RNA splicing (*13*).

Like group I self-splicing, self-splicing of the mitochondrial *Saccharomyces* group II intron *bI1* occurs by a two-step transesterification mechanism (*17–19*). The excised lariat intervening sequence (IVS) RNA acts as a ribozyme by catalyzing transesterification reactions with multiple turnover on ligated-exon RNA substrates in trans (*20–23*). This general recombinase and 3′ terminal transferase activity of the group II lariat IVS is illustrated in Fig. 1A. Selection of the donor and acceptor RNA, and thereby specification of the transesterified

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