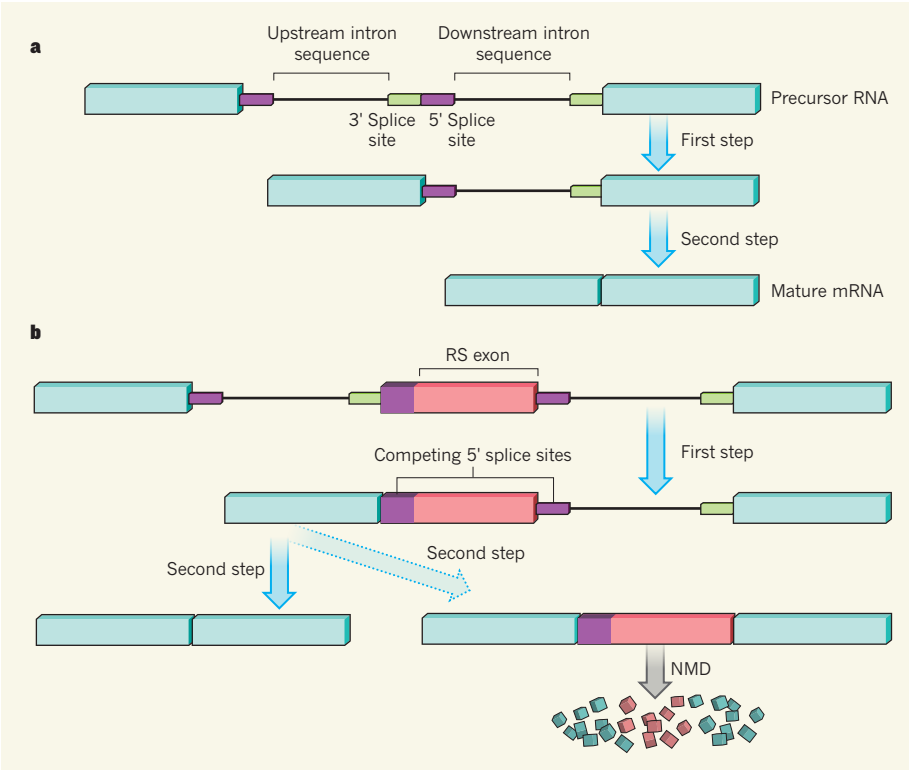

Figure 1 | Mechanisms of recursive splicing. **a**, In recursive splicing, long intron sequences of precursor RNA are removed in a stepwise process mediated by juxtaposed internal 3′ and 5′ splice sites. In the first step, the 3′ splice site is used to remove the upstream intronic sequences. The second step uses the 5′ splice site to remove the downstream intron sequences, forming a mature messenger RNA. Duff *et al.*[5] report that this recursive splicing process occurs in the fruit fly *Drosophila melanogaster* much more commonly than was previously thought. **b**, Sibley *et al.*[6] find that some recursively spliced messenger RNAs — including all those known in humans — contain a recursive splicing (RS) exon. The RS exon can be either completely removed or retained in the mature mRNA, depending on which of two competing 5′ splice sites is used in the second step. Most mRNAs that harbour RS exons are degraded by nonsense-mediated RNA decay (NMD).

proteins[7]. Second, it provides opportunities for quality control: RS exons are almost always spliced out of normal mRNAs, but the authors found that they are usually retained when the upstream exon is generated from an aberrant promoter sequence or from a potentially faulty splicing event. RS-exon inclusion is favoured in these instances because its 5′ splice site drives splicing more effectively than the 5′ splice site required to remove the RS exon.

RS-exon retention often leads to death of the mRNA, because RS exons typically contain in-frame premature-termination codons — sequences that cause the mRNA to be degraded by the nonsense-mediated RNA decay (NMD) pathway[8] (Fig. 1b). This is physiologically relevant because most RS-exon-containing mRNAs are probably 'garbage' transcripts. But a subset of these mRNAs may be functional; their formation might be induced when NMD is repressed, such as during particular stages of development and in response to stress[8].

Why do humans and *Drosophila* seem to use different mechanisms to splice out recursive exons? Species-specific splicing factors may be one explanation. Alternatively, differential RS-exon usage might result from known differences in how these two species define splice sites[7]. It could also be that the differences in these two species seem greater than is actually the case — for example, RS exons might participate in an intermediate step of *Drosophila* recursive splicing, being included in mature RNAs so infrequently that they are usually undetectable.

It was previously proposed that recursive splicing might increase the fidelity of splicing[1–3]. Sibley *et al.* examined this possibility using antisense oligonucleotide molecules to block recursive splice sites. They found that this had no obvious effect on the recursive splicing of two human genes, and only modestly inhibited recursive splicing of a zebrafish gene. These data suggest that recursive splicing is not required for the efficiency or accuracy of long-intron splicing. It is possible, however, that this experiment did not reveal a crucial role of recursive splicing because blockade of the natural recursive splice site led to the use of other recursive splice sites that are not normally used.

Duff *et al.* performed extensive genome-wide analyses of *Drosophila* (35 dissected tissues, 24 cell lines and 30 developmental stages) and found that recursive splicing occurs in about 6% of long introns in all tissues tested. By contrast, recursive splicing may exhibit some tissue specificity in humans. Sibley *et al.* found that genes with long introns tend to be expressed in the human nervous system, and they identified recursively spliced RNAs expressed in the human brain[6]. Duff *et al.* detected some selectivity for recursive splicing in the brain in a screen of 20 human tissues (including fetal brain and adult cerebellum), but this may partly reflect the difficulty of detecting recursively spliced RNAs in tissues that express such RNAs at low levels. It will be important to determine whether this specificity, if real, results from the tendency of recursively spliced genes to be expressed in the brain, or whether cells in the nervous system have factors that promote recursive splicing.

Many genes that have long introns, including those that undergo recursive splicing, are linked to neurological diseases and to autism[9–11]. Whether these conditions are sometimes triggered by errors in the multi-step recursive RNA-splicing process will be an exciting avenue for future studies. ■

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CLARIFICATION

The News & Views article 'Quantum physics: Two-atom bunching' by Lindsay J. LeBlanc (*Nature* **520,** 36–37; 2015) described a paper reporting a type of two-particle quantum interference called the Hong-Ou-Mandel effect using helium-4 atoms, but did not make clear that similar two-particle quantum interference had previously been reported using rubidium-87 atoms (A. M. Kaufman *et al. Science* **345,** 306–309; 2014).