

experiments blind and generally using poor experimental design were all flaws in ethological work of that early period.

How did you become interested in animal welfare? An animal in pain or distress is not a suitable subject for the study of normal behaviour. The animal's welfare and state of well-being were, therefore, important to ethologists and the undoubted ethical considerations added another dimension. I was tempted by a request from the National Trust to examine the welfare of deer hunted with hounds in the West of England. Though I had done nothing like this before — indeed nobody had — I thought it would be an interesting challenge. I had an able research associate in Liz Bradshaw and after a year and a half we reported to the Council of the National Trust, concluding that the level of total suffering of red deer would be markedly reduced if hunting with hounds were ended. Hunting red deer with hounds could no longer be justified on welfare grounds, given the standards applied in other fields such as the transit and slaughter of farm animals and the use of animals in research.

On the strength of our report, the National Trust immediately banned hunting of red deer with hounds on its land. Our study was the first of its kind and was understandably controversial because it challenged the way of life of people living in stag-hunting country. They were determined to show that we were wrong and commissioned a new study; however, the new group obtained gratifyingly similar results to those obtained in our work, even though they had no interest in its welfare implications.

In another foray into animal welfare, I conducted a survey of the breeding of pedigree dogs. Breeding for exaggerated characteristics like a flattened face or sloping back clearly had welfare implications. So did close inbreeding conducted in the interests of retaining desired characteristics. My report was critical of what some breeders were doing, but this time it was like pushing on an open door and I received none of the odium that accompanied my report on stag-hunting.

Has any theme run through your academic work? I have often attacked the distinction that is

drawn in describing behaviour as being either innate or acquired. The study of imprinting which occupied much of my research life provides an important insight into the development of behaviour. The bird has strong predispositions to respond socially to particular things and its capacity to develop preferences generates a robust outcome. Therefore, opposing robustness and the consequences of plasticity provides a misleading picture of what happens in development. For that reason I have advocated that processes of development should be the object of study. This is now part of the broader study of the field known as epigenetics. In an attempt to understand the process, I was involved for many years in collaboration with neurobiologists, particularly Gabriel Horn. This work was crowned with identifying a crucial structure in the brain which is closely involved in imprinting. Epigenetics involves the active involvement of the individual in its own development. Shortly after hatching birds such as ducklings work to present themselves with an object with which they can be imprinted. The behaviour that leads a bird to an imprinting stimulus is reinforced, but the learning process is different from imprinting.

I retained an interest in the whole animal and the active role of its behaviour in development. Much of the work with my students and associates was on play in mammals. In writing about this in a recent book with one of my former students, Paul Martin, we concluded that play enables an individual to cope creatively with novel challenges later in its life. An individual's activities not only affect its own development, they also impinge on the environment of its descendants. This provides one of the links between epigenetics and evolution. Bridging the gap between seemingly different questions that may be asked about behaviour is a trend that would have excited Niko Tinbergen. He was clear about the difference between the questions but also saw the value of bringing them together. That synthesis remains as important a contribution of ethology as it did in his day and has run as a theme throughout my academic life.

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Quick guide

Spliceosomes

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What are spliceosomes?

Spliceosomes are huge, multimegadalton ribonucleoprotein (RNP) complexes found in eukaryotic nuclei. They assemble on RNA polymerase II transcripts from which they excise RNA sequences called introns and splice together the flanking sequences called exons. This so-called pre-messenger RNA (pre-mRNA) splicing is an essential step in eukaryotic mRNA synthesis. Every human cell contains ~100,000 spliceosomes, which are responsible for removing over 200,000 different intron sequences. Human cells contain two types of spliceosome: the major spliceosome responsible for removing 99.5% of introns and the minor spliceosome, which removes the remaining 0.5%.

What are spliceosomes made of?

Spliceosomes contain both proteins and RNAs. Yeasts have ~100 spliceosomal proteins, whereas over 300 different proteins associate with human spliceosomes (Figure 1A). Many of these proteins have specific RNA recognition activities, while others are NTPases that function to drive the overall process forward and ensure its fidelity. Numerous other proteins bind stably to small nuclear RNAs (snRNAs) to form small nuclear RNPs (snRNPs, pronounced 'snurps'). Major spliceosomes are assembled from U1, U2, U4, U6, and U5 snRNPs (which are named according to the U snRNA(s) they contain); minor spliceosomes are assembled from U11, U12, U4atac, U5, and U6atac snRNPs (Figure 1B).

How did the various spliceosomal parts get their names?

The U snRNAs were originally discovered as abundant small uridine-rich RNA molecules present in mammalian nuclei, and they were initially numbered in order of their apparent abundance. U1, U2, U4, U5, U6, U11, and U12 were later found to be spliceosome components. U7 snRNA is required for histone mRNA 3'-end processing; the other abundant

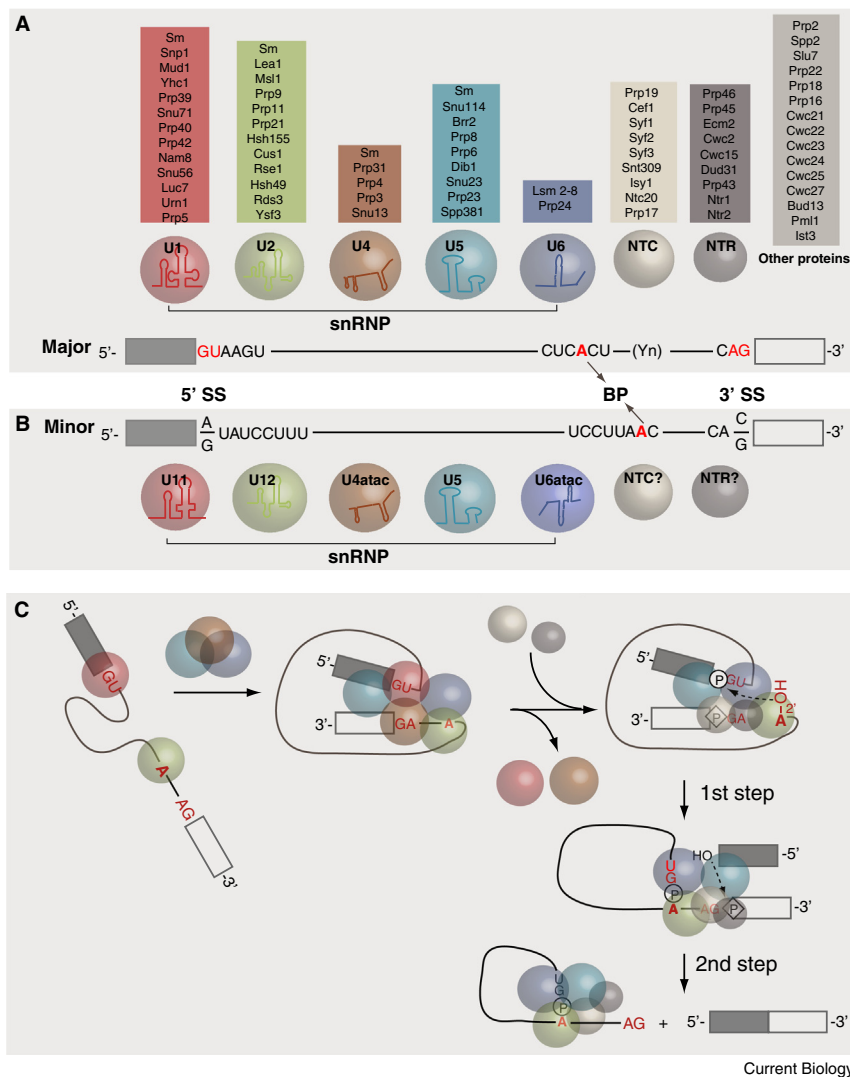


Figure 1. Spliceosome components and assembly. (A) Protein and snRNA composition of yeast spliceosomes (akin to the ‘major’ metazoan spliceosome). Also shown are the conserved sequences of major (A) and minor (B) introns (solid line) with exons (boxes), and intronic consensus sequences (most highly conserved nucleotides in red). NTC, nineteen complex; NTR, nineteen complex related. SS, splice site; BP, branch point. ‘Sm’ indicates the seven Sm proteins, i.e. B, D1, D2, D3, E, F, and G; ‘Lsm’ indicates the seven Lsm proteins, i.e. Lsm2, Lsm3, Lsm4, Lsm5, Lsm6, Lsm7, and Lsm8. (B) Components of the minor spliceosome. snRNPs contain the indicated snRNAs plus stably bound proteins, many of which are shared with the major spliceosome. NTC and NTR association with the minor spliceosome has only been inferred to date. (C) Simplified spliceosome assembly scheme showing the two chemical steps of splicing. See text for details.

U snRNAs (U3, U8, U9 and U10) are all involved in ribosome biogenesis. U4atac and U6atac are much less abundant than other spliceosomal snRNAs, so were only discovered and named when it was realized that there must be other snRNAs that recognize the minor intron class. The first and last two DNA nucleotides of minor introns are most often AT and AC, respectively (Figure 1B), hence the names U4atac and U6atac.

Many spliceosomal proteins have PRP names, e.g. Prp2, Prp5,

Prp8, etc. (Figure 1A). In yeast, mutations in these genes lead to ‘pre-mRNA processing’ defects. Confusingly, orthologous genes can have different PRP names in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* because the original mutational screens were performed around the same time and a unified naming system has yet to be devised. Other core spliceosomal proteins include CWC (complexed with CDC5), CWF (complexed with CDC five), SPF

(sensitivity to *Pichia farinosa* killer toxin), SYF (synthetic lethal with cdc40). The nineteen complex (NTC) is a large protein-only subcomplex named after its most abundant component, Prp19, while another small protein-only complex known as NTR (nineteen complex related) contains factors involved in spliceosome disassembly.

Some major spliceosomal proteins were first discovered in vertebrates. The seven Sm proteins, which form a ring encircling a specific binding site in almost all spliceosomal snRNAs, were named after the patient (Smith) with whose autoimmune antibodies they react. A similar set of proteins (Lsm, for ‘like Sm’) were later found to encircle U6 and U6atac snRNAs, the only two spliceosomal snRNAs lacking a consensus Sm-binding site. Two additional large classes of metazoan splicing factors are the hnRNP proteins, so-called because they are found associated with heterogeneous nuclear RNA (hnRNA), and the SR proteins, named for a carboxy-terminal domain rich in arginine-serine (RS) dipeptides.

How does the spliceosome do its job? Spliceosomes must excise non-coding introns from precursor transcripts and stitch the flanking exons back together to create mature spliced mRNAs. To do so, the splicing machinery assembles in a stepwise manner on the ends of introns, with U1 snRNP recognizing the beginning of an intron (5’ splice site, the donor site) and U2 snRNP recognizing a feature (the branch site) at the other end in the vicinity of the 3’ splice site (acceptor site). After numerous structural rearrangements that involve both the addition of new components and the ejection of many others, splicing occurs in two chemical steps: firstly, cleavage at the 5’ splice site coupled to formation of a lariat structure in which the first nucleotide of the intron is linked via a 2’–5’ phosphodiester bond to the branch site adenosine; and secondly, ligation of the two exons, coupled to cleavage at the 3’ splice site (Figure 1C). The spliceosome then disassembles from the excised intron, which is subsequently debranched and degraded.

How do spliceosomes affect gene expression? Because the vast majority of protein-coding genes in humans contain introns (typically 9 or 10, but some have more than 100!), splicing is an essential step in gene expression. High-throughput sequencing has now revealed that ~95% of human genes are also subject to alternative splicing, which allows for the synthesis of many different mRNAs from a single DNA gene. By encoding alternative protein isoforms or harboring different regulatory sequences in their untranslated regions, alternatively spliced mRNAs greatly enhance biological complexity. The act of splicing itself also has important consequences for gene expression beyond intron removal. By stably depositing on exons proteins that accompany mRNPs to the cytoplasm (e.g. the exon junction complex, EJC), splicing can affect the subcellular localization, translation efficiency and decay kinetics of the mRNA. In particular, mRNA decay driven by EJC location relative to the stop codon is a crucial mediator of cellular protein abundance.

Are spliceosomes associated with any diseases? Many human diseases are caused by either mis-splicing of a single gene or mis-regulation of the entire spliceosome. Around 35% of human genetic disorders are caused by a mutation that alters the splicing of a single gene. Such mutations can add/remove a single splice site (e.g., α - or β -thalassemia) or shift the balance of alternative splicing by affecting the inclusion/exclusion of a cassette exon (e.g., frontotemporal dementia driven by tau mis-splicing). Some mis-splicing events generate an mRNA isoform that is subject to rapid degradation. Single point mutations that affect splicing can thereby result in large changes to both protein structure and protein abundance. Other diseases are caused by mutations in the splicesomal proteins themselves, thereby affecting splicing of many transcripts. For example, mutations in several core spliceosomal proteins (e.g., Prp8, Prp3, Prp31, and Brr2) have been shown to cause autosomal dominant retinitis pigmentosa. Mutations in splicing

factor 3B subunit 1 (SF3B1) and U2 auxiliary factor 35 (U2AF35) are frequently associated with chronic lymphocytic leukaemia and myelodysplasia. Other cancers are associated with mis-regulation of splicing factor levels. Therefore, the spliceosome has recently emerged as a new target for the development of novel anti-cancer therapies.

What remains to be explored? Because of its highly dynamic and complex nature, an atomic level structure of the spliceosome remains an elusive goal. Nonetheless, much progress has recently been made by crystallizing subsets of spliceosomal components, including U1 and U4 snRNPs and the central core protein Prp8. Other major questions regard the exact molecular mechanisms by which spliceosomes achieve high splicing accuracy, while simultaneously allowing for flexibility in splice site choice to permit alternative splicing. To answer these questions, new tools such as single-molecule microscopy, bioinformatics, and high-throughput methods for determining protein–protein, protein–RNA and RNA–RNA interaction dynamics are increasingly being developed and applied.

Where can I find out more?

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Primer

Plant grafting

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Since ancient times, people have cut and joined together plants of different varieties or species so they would grow as a single plant — a process known as grafting (Figures 1 and 2). References to grafting appear in the Bible, ancient Greek and ancient Chinese texts, indicating that grafting was practised in Europe, the Middle East and Asia by at least the 5th century BCE. It is unknown where or how grafting was first discovered, but it is likely that natural grafting, the process by which two plants touch and fuse limbs or roots in the absence of human interference (Figure 3), influenced people's thinking. Such natural grafts are generally uncommon, but are seen in certain species, including English ivy. Parasitic plants, such as mistletoe, that grow and feed on often unrelated species may have also contributed to the development of grafting as a technique, as people would have observed mistletoe growing on trees such as apples or poplars.

Today, plant grafting is widely used in orchards, greenhouses, vineyards and gardens. One common application is grafting the shoot of one plant, termed the scion, to the root of a different plant, termed the rootstock, to increase or decrease the size of the plant. Alternatively, grafting can improve stress resistance or allow plants to grow in new environments. Plant grafting has also been important for the discovery of proteins, RNAs and hormones that act over long distances. This Primer summarises the mechanisms of graft formation, discusses why some plants graft whereas other do not, and describes how grafting is important for agriculture and for scientific research.

The mechanism of graft formation Although grafting has been practised for over 2500 years, ancient texts often gave confusing and contradictory information about what plants could be successfully grafted together, known as compatible grafts, and which plants could not, known as incompatible