A dual role for RNA splicing signals

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Gene expression in eukaryotes requires the excision of introns from pre-mRNAs by splicing, which eliminates intervening sequences that interrupt the translation open-reading frame in mRNAs. The spliceosome—a complex of small RNAs and proteins—recognizes and acts on sequences at the exon–intron junctions—5'-splice site [SS] and 3'-SS—and at the branchpoint (Fig 1). Splicing is traditionally considered to be highly accurate and efficient because of the spliceosome's specific recognition of these three splicing signals. However, there is growing evidence that the splicing of many pre-mRNAs is sub-optimal and that unspliced precursors can be detected after inhibition of RNA degradation activities (He *et al*, 1993; Jaillon *et al*, 2008; Mitrovich & Anderson, 2000; Sayani *et al*, 2008). These observations bring to light the concept that quality control mechanisms exist to eliminate RNA molecules that have escaped the splicing process.

In the absence of RNA quality control, unspliced RNAs would accumulate and could be translated at high efficiency. Because intronic sequences are not usually constrained to preserve the open-reading frame in unspliced RNAs, there is a high chance of the occurrence of a premature stop codon in the intron. Translation of unspliced RNAs would thus generate truncated polypeptides with potential dominant-negative functions and deleterious consequences for cellular pathways. Nonsense-mediated mRNA decay (NMD) is an RNA turnover mechanism that eliminates transcripts containing premature termination codons (Isken & Maquat, 2007) and thus provides an efficient mechanism to degrade most unspliced RNAs if they reach the cytoplasm. Indeed, NMD has been implicated in discarding a large number of unspliced mRNAs (Farlow *et al*, 2010; He *et al*, 1993; Jaillon *et al*, 2008; Mitrovich & Anderson, 2000; Sayani *et al*, 2008). Elimination of unspliced RNAs by NMD might also have contributed to intron

gain during the evolution of genomes, as it minimizes the negative impact of inefficiently spliced transcripts (Farlow *et al*, 2010).

The ability of NMD to degrade unspliced RNAs relies on the presence of a stop codon that is recognized as premature (Isken & Maquat, 2007). Strikingly, intronic signals recognized by the spliceosome to mediate RNA splicing also coincide with sequences that have the potential to trigger translation termination (Fig 1). For instance, one of the two optimal 3'-SS sequences, UAG, corresponds to a translation termination codon (Fig 1). The typical branchpoint sequence in fungi UACUAAC also contains a UAA stop codon (Fig 1). Recent work on the yeast *Yarrowia lipolytica* has shown that this phenomenon is widespread and that all three splicing signals in this species (5'-SS, branchpoint and 3'-SS) contain sequences that have the potential to induce translation termination if the introns are inserted into a particular frame (Mekouar *et al*, 2010). This includes the consensus 5'-SS GUGAGU (Fig 1), which differs from that of other fungi and includes a UGA stop codon. The presence of a stop codon in the 5'-SS is also prominent in other species such as *Drosophila* (Farlow *et al*, 2010). Thus, all

three splicing signals can potentially mediate premature translation termination and NMD degradation. This prominence of stop codons within splicing signals provides a way to ensure the cytoplasmic degradation of unspliced RNAs by NMD, regardless of the remainder of the intronic sequence.

These observations suggest that the splicing signals have been specifically selected or have co-evolved with the translation machinery to maximize the likelihood of degradation of unspliced or aberrantly spliced RNAs by NMD. The fact that splicing signals coincide with stop codons was noted previously (Senapathy, 1988), but was interpreted as evidence that splicing signals originated from stop codons. In the light of the widespread involvement of NMD in degrading unspliced precursors, and its potential evolutionary impact on genome building (Farlow *et al*, 2010), it is striking to consider that splicing signals play a dual role in the life and death of RNA molecules. They are essential to generate correctly spliced RNAs, but also to mediate the destruction of RNAs that have escaped the splicing machinery. Whether this is a coincidence or reflects a more direct evolutionary relationship remains to be established.

Fig 1 | Prominence of translation termination codons (red) within splicing signals. Exons are boxed, the intron is shown as a blue line. Intronic sequences that coincide with stop codons are indicated in red and highlighted by a stop sign. Consensus sequences at the 5'-splice site, 3'-splice site and branchpoint are shown. The consensus sequence from the 5'-splice site is from *Yarrowia lipolytica*.

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Anthropomorphism in science

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It is a common characteristic of our species to assign human emotion and behaviour to other creatures and even inanimate objects—just ask any car owner. Common examples of such anthropomorphisms involve animals and pets, especially dogs and cats. These domesticated species are sometimes considered to behave like us and think like us, becoming pseudo-human.

One might think that such sentimental anthropomorphism would be unlikely to spill over into the biological sciences, but this is not so. Microbiology seems particularly susceptible and the literature is littered with examples of bacteria having to ‘make a choice to use a particular substrate’ or a ‘decision to make a compound’ and even ‘needing something’. When bacterial conjugation was discovered in the 1950s, bacteria were even classified as males and females participating in sexual mating. I am sure that many of you will be able to come up with examples from other fields.

I would argue that in a number of instances anthropomorphic thinking has misdirected biological enquiry. It is often assumed that microbes in their natural environments are in a constant war of attrition for space and nutrients. Many publications speak of battlefields and the production of chemical weapons to permit one or more organisms to successfully exploit a particular environment. Does ascribing human militaristic means and ends to bacteria make sense? There is enormous diversity in microbial phyla and the biosphere is an extraordinarily complex collection of distinct organisms. A given soil sample might contain 10^9 microbes per gram with a thousand or more species living happily together (an

anthropomorphic statement if ever there was one). In the human gut, microbes number many trillions with upwards of 1,000 phylotypes; are they all engaged in lethal conflict with each other? Despite the fact that small molecules with antibiotic activity can be isolated from gut bacteria grown in the laboratory, there is no *in situ* evidence that they actually play such roles in the intestinal tract; it is equally likely that these molecules are mediating interactions with mucosal cells lining the gut. However, our ignorance of the workings of microbial communities in these environments is profound and remains tainted by our anthropomorphism.

The isolation of antibacterial small molecules from natural sources has completely changed the face of infectious disease treatment. Not surprisingly, the antibiotic activities of these compounds were assumed to be their natural roles and they were labelled on the basis of militaristic function. Yet, there is increasing support for the notion that these compounds play many different roles as modulators of transcription and physiological regulators/signalling agents. In all probability, many other examples of anthropomorphic and anthropocentric thinking have biased biological studies; although no one has yet thought to claim that willow trees produce aspirin just for our benefit!

Humans seem able to make a ‘pet’ of almost anything; researchers working with *E. coli* or *S. cerevisiae* might well develop an affinity for their subjects. It has even been reported that microbiologists in a German sewage plant play Mozart to their hard-working microbes to enhance their efficiency in biodegradation. Given that humans harbor large numbers of bacteria and that they

are integral to so many of our functions, is it so strange to imagine that microbes might behave like us? My tongue-in-cheek comments can best be given serious consideration in the light of the proposal that the human gut flora are a forgotten organ (O’Hara & Shanahan, 2006); they are a critical part of the whole.

If we accept the essential roles of microbes in our evolution and existence, should they be afforded some rights akin to UN charters? After all, I assume that we are concerned about protecting all living things; except, of course, malicious creatures like mosquitoes. Bacteria are essential to the life of every eukaryotic organism on Earth; without bacteria we would have defective immune systems, malfunctioning digestive systems and no plants or flowers. The bacterial denizens of humans are certainly not new players, but it is only recently that science has demonstrated their critical roles.

Some thoughtful people have taken the question of bacterial rights seriously. In 2004, Charles Cockell proposed that microbial communities and ecosystems should be protected. He argued that since microbes enable all other life forms, they should have some constitutional rights. Whether this proposal is reasonable and achievable is a topic for discussion, although I think the suggestion is ridiculous. Nevertheless, I am concerned that given the universality of bacterial–eukaryote interdependence, biological conservation efforts continue to ignore the microbial world. Diversitas and similar biodiversity programmes barely mention bacterial diversity in their manifestos. Why protect insects and not microbes? Conversely, it is hard to make a case for the rights of deadly human pathogens, but then again, they don’t seem to need our protection.

The burgeoning knowledge of the vital importance of microbes to all things human will undoubtedly have a considerable influence on the treatment of many diseases. Yet, however fond or fearful we are of bacteria, we must accept that bacteriophages—the most abundant life forms in the biosphere—generally wipe out around 50% of the world’s bacterial population every few days, so why worry?

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