Splicing in 4D

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Science **338**, 1547 (2012);
DOI: 10.1126/science.1233219

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

was their prior work together in the biopharmaceutical sector. Since the late 1990s, the governments of Brazil and Cuba had been steadily promoting scientific interaction to emphasize South-South collaboration. This sector was singled out for partnerships given each nation's strengths in this arena as well as some common health problems such as tropical diseases and a growing noncommunicable disease burden (including cardiovascular disease and cancer). Together, they had an applied focus—to increase the availability of affordable health products that serve local health needs. Bio-Manguinhos had already collaborated with several Cuban biotechnology institutes and, for example, transferred affordable technologies from Cuba to produce interferon alpha-2b and erythropoietin. The main benefits of these joint projects were savings for Brazil's public health system and income from royalties for Cuba. These earlier, mutually beneficial collaborations were a strong foundation to build upon.

The Brazil-Cuba meningitis project was not their only collaboration to have benefited a third party. They are now jointly promoting health and development in Haiti following the 2010 earthquake and will construct hospitals, support immunization programs, and strengthen laboratories for disease surveillance in Haiti (*11*).

Most international collaborations include elements of self-interest and desires for the alliance to benefit all parties concerned. Sometimes developing countries are transparent about this and "tie" their aid by demanding that products and services be supplied exclusively by them instead of being purchased on the international market. China and India, for example, tie their aid to Africa by requesting that services and products in their African initiatives be supplied by them (*12*, *13*). However, South-South and North-South approaches to assistance can be viewed as having different philosophies. Whereas the latter is generally grounded in altruism, the former is built on solidarity between countries that have had to survive under challenging conditions. This distinction sets a different tone for South-South collaboration. The solidarity among developing countries began to surface in the 1950s during the quest for independence from colonial powers, and many developing nations sought alternatives to dealing with the North to address issues of concern. Today, there is an increasing scope for South-South interaction, as the developing world becomes technologically proficient and experiences economic growth (*14*). South-South partnerships are therefore promising for tackling many shared challenges in health, agriculture, and environmental protection. Given that aid from traditional Northern donors is declining with the continuing global economic recession (*15*), international and philanthropic organizations, and governments in high-income countries, should recognize South-South enterprises to a larger extent in strategies that promote global health and development.

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Acknowledgments: Supported by the International Development Research Centre and Genome Canada through the Ontario Genomics Institute, and a New Investigator Award (H.T.) of the Canadian Institutes of Health Research.

10.1126/science.1233318

EVOLUTION

Splicing in 4D

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Flexibility in regulating RNA splicing can generate diverse phenotypic differences among equivalent organs across vertebrates.

In the chapter of *The Origin of Species* entitled "Difficulties on Theory," Charles Darwin found it "most difficult to conjecture by what transitions an organ could have arrived at its present state." On pages 1587 and 1593 of this issue, Barbosa-Morais *et al*. (*1*) and Merkin *et al*. (*2*) advance our understanding of the molecular mechanism by which the genome generates differences in organs between species. This part of the answer relies on the broken syntax of genomic messages and uncovers striking differences in how evolution shapes the different layers of gene regulation.

Genes in eukaryotic organisms are first transcribed as precursor messenger RNAs (pre-mRNAs) in which "meaningful" sequences (exons), which code for amino acids or harbor regulatory sequences, are interrupted by (usually) longer pieces (introns) that are removed by splicing. The resulting mature mRNAs are then translated into proteins, which carry out enzymatic and structural functions in the cell. Remarkably, different cell types can interpret the same sequence of a pre-mRNA either as an exon or as an intron. This leads to different patterns of splicing that represent cell type–specific alternative interpretations of the genomic information. Alternative splicing allows the shuffling of protein-coding domains or confers distinct sensitivity of the spliced mRNAs to regulatory factors (*3*). Thus, gene transcription and alternative splicing provide separate mechanisms by which particular cell types can determine the complement of proteins required for carrying out their specialized functions in the organism.

Patterns of tissue-specific gene activation are highly conserved among vertebrates. Indeed, Merkin *et al*. find that only when considering long evolutionary periods (e.g., 300 million years after the split between birds and mammals) can a species-specific signa-

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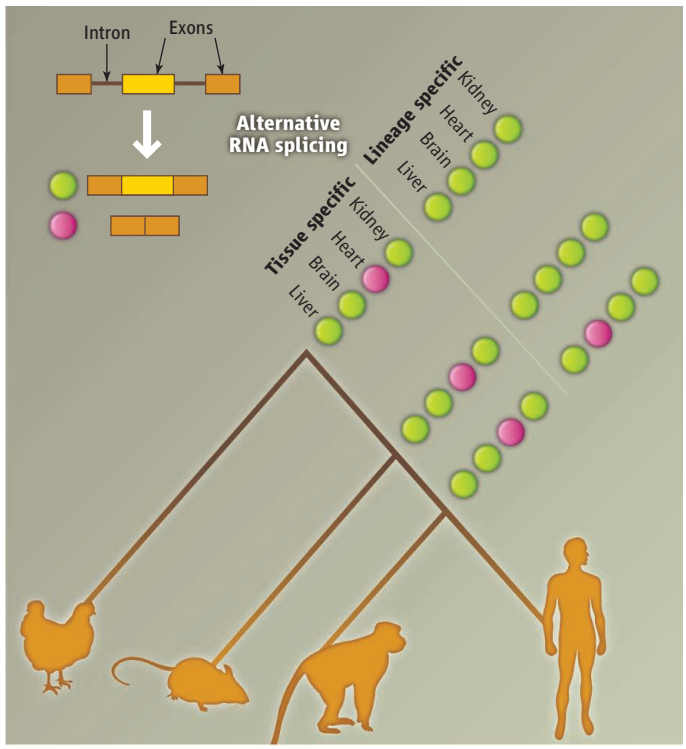
ture of gene transcription be seen to dominate over the highly conserved tissue-specific signatures. What could then be the source of genome-originating divergence leading to differences in organs between species?

Previous comparisons indicated that conservation of alternative splicing patterns in orthologous genes is limited (*4*). For example, only 10% of exons conserved between mouse and human display alternative splicing in both species (*5*). The general lack of phylogenetic conservation has been considered an argument against the functional relevance of most alternative splicing events. The results of Barbosa-Morais *et al*. and Merkin *et al*., however, bring a fresh perspective to this issue because both studies reveal that alternative splicing and transcription regulation are under very different evolutionary constraints. Indeed, these studies find that alternative splicing patterns are dominated by species-specific differences that accumulate even during relatively short evolutionary periods of 6 million years, implying that tissue-specific splicing diverges in particular lineages at a pace one to two orders of magnitude faster than transcriptional changes (see the figure).

Nevertheless, Barbosa-Morais *et al*. and Merkin *et al*. uncovered a group of a few hundred alternatively spliced exons whose tissue-specific regulation is highly conserved, in some cases over periods of hundreds of millions of years. The unprecedented resolution of the high-throughput RNA sequencing data provides a wealth of information that, together with results from other technologies (*6*), will add to our appreciation of the diversity and potential functions of splicing regulation in organogenesis.

The complex molecular machinery of the spliceosome recognizes the sequence boundaries between exons and introns and mediates intron removal (*7*). Regulation of splice site choice involves the interplay between numerous regulatory sequence motifs present in introns and exons and the trans-acting factors that recognize these sequences to promote or prevent spliceosome assembly on particular splice sites (*8*, *9*). Merkin *et al*. discovered enrichment in binding sites for well-known regulators of alternative splicing during cell differentiation, uncovering an ancestral splicing code.



Splicing and species diversification. Although conserved tissue-specific patterns of alternative exon usage exist, most differences in alternative splicing patterns are distinct between evolutionary lineages and can contribute to phenotypic divergence of organs in different vertebrate species.

Shortly after the discovery of introns, the molecular biologist Walter Gilbert speculated that the "mosaic" intron-exon architecture of genes could accelerate evolution (*10*). Gilbert reasoned that incremental mutational steps near the intron-exon boundaries could lead to the coexistence of alternative transcripts and therefore allow exploration of sequence space without radically disrupting the previous gene function. The results of Barbosa-Morais *et al*. and Merkin *et al*. support this concept as a general evolutionary strategy adopted by vertebrates, reporting frequent conversion between alternative and constitutive splicing and also an increase in the prevalence of alternative splicing in primates. This plasticity can be attributed to evolutionary tinkering with regulatory sequence motifs, as demonstrated by Barbosa-Morais *et al*. using mouse cells engineered to harbor human chromosome 21. In these cells, human transcripts maintain their distinct patterns of alternative splicing despite being produced by the mouse splicing machinery. The regulatory flexibility and constant flux of alternative splicing exploits the combinatorial interplay and positional effects of a relatively small number of generally ubiquitous splicing regulators (*8*, *9*). This is again in contrast to tissue-specific transcription, which relies on a larger set of tissue-specific activators (*8*).

Barbosa-Morais *et al*. and Merkin *et al*. showcase ways in which vertebrates can capitalize on the potential of split gene organization for adding, removing, or altering specific aspects of transcript functionalities. Merkin *et al*. provide a compelling example in the control of protein phosphorylation: Exons that are differentially included among tissues encode protein segments enriched in phosphorylation sites. Differential inclusion of these exons is more prominent in those tissues that express the enzymes (kinases) that phosphorylate these sites, suggesting that differential splicing—rather than kinase amounts—predominantly modulates the extent of protein modification. Enrichment in phosphorylation sites is also observed in alternative exons with highly variable tissue distribution among species, implying a role for alternative splicing in the modification of kinase signaling circuits between species.

Barbosa-Morais *et al*. additionally indicate that species-specific splicing is frequent in regulatory gene encoding nucleic acid binding proteins, and that both tissue- and species-specific exons typically correspond to unstructured regions of proteins that are often involved in protein-protein interactions (*11*, *12*). Together, the two studies underscore the multidimensional impact of alternative splicing by modulating the scope of signaling, gene regulation, and protein-protein networks both in tissue differentiation and during evolution.

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10.1126/science.1233219

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