SYSTEMS BIOLOGY

Protein isoforms: more than meets the eye

Alternative splicing imparts distinct functions through isoform-specific protein–protein interactions.

The alternative splicing of transcripts is known to contribute to the diversity of the proteome by allowing single genes to produce several distinct protein isoforms. In some extreme cases the functions of two isoforms of the same protein can have opposing effects on a cellular process (cell survival versus death, for example), but most translated splice variants are thought to function in closely related biochemical pathways. To get a better handle on the functional consequences of alternative splicing on a global scale, Marc Vidal of Harvard University, Yu Xia of McGill University, and Lilia Iakoucheva of the University of California, San Diego, led an effort to systematically compare differences in protein–protein interactions for splice-variant isoforms of several hundred human genes.

The researchers first selected 1,500 protein-coding genes and used specific primers encompassing annotated start and stop codons to amplify and clone 'reference' and 'alternative' open reading frames (ORFs) from five human tissues. Those sequences were then transferred to a cloning pipeline allowing downstream expression and functional analyses. A total of 398 reference and 637 alternative ORFs were systematically tested for binary interaction in a stringent yeast two-hybrid screen against 15,000 unique clones (human ORFeome v5.1). From the detectable interactions between reference ORFs and their splice variants, three classes emerged: variants with identical (21%), overlapping (63%), or completely distinct (16%) sets of interaction partners. In most cases, the difference in interaction patterns between isoforms could be traced to the inclusion or exclusion of short linear motifs.

Network-based analyses further indicated that differences in interaction properties between protein isoforms can be as great as those between proteins encoded by different genes. Given that splice isoforms often exhibit tissue-specific expression, the functions of a single gene in a given tissue could thus be due to the formation of tissue-specific protein complexes. The researchers also performed preliminary cross-species complementation assays, which supported distinct activities for different isoforms and led them to conclude that a significant fraction of splice isoforms are likely to encode 'functional alloforms'.

The reported results have far-reaching implications, revealing a layer of complexity whereby a single gene can produce proteins with distinct functions in different cell types.

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RESEARCH PAPERS

Yang, X. *et al*. Widespread expansion of protein interaction capabilities by alternative splicing. *Cell* **164**, 805–817 (2016).