

Embracing the complexity of RNA regulatory networks – layer by layer

Commentary on:

“Multilayered Control of Alternative Splicing Regulatory Networks by Transcription Factors”. Han, H, Braunschweig, U et al. (2017) *Molecular Cell* **65**, 539-553

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The alternative splicing (AS) of precursor mRNA is tightly regulated and serves to generate a vast repertoire of RNA and protein variants and to enable expression in a cell-specific manner¹. Furthermore, AS profoundly expands the functional capacity of the genome by impacting other regulatory layers, including mRNA transcription, turnover, transport, and translation. Equally, factors regulating chromatin and transcription complexes influence AS. This extensive crosstalk dictates the need to develop a multidimensional understanding of how AS is controlled.

AS critically underlies a range of diverse biological processes, such as cell fate determination², and is frequently mis-regulated in disease; RNA mis-splicing accounts for up to 15% of all inherited diseases and mutations affecting splicing comprise 50-60% of all disease-causing mutations³. To exemplify relevance to the cardiovascular system, the dynamic adaptation during perinatal cardiac growth requires sarcomere components, such as titin, to undergo an isoform switch from foetal to adult type in order to adjust ventricular filling⁴. Not surprising, therefore, is the association of these genes with congenital cardiomyopathies⁵, nor their role in pathophysiological adaptation. In animal models of HFpEF, increased chamber stiffness was associated with elevated expression of the shorter, less compliant N2B titin isoform⁶; conversely, in patients with end-stage systolic failure⁷, up-regulation of the more compliant titin isoforms occurs, as a compensatory mechanism to correct for myocardial stiffening induced by altered matrix composition. Loss-of-function mutations in human RBM20, a splicing regulator, were found to cause a DCM-like phenotype due to persistent expression of a giant foetal titin isoform (N2BA-G) in adult heart⁸.

A very relevant application in our field, given the complex AS patterns that govern cell fate specification and maturation, is towards cardiac regenerative medicine; whether by stem cell transdifferentiation, direct reprogramming, activation of resident progenitors or evoking cell cycle re-entry, the underlying molecular and cellular mechanisms are under the control of integrated transcriptional and AS programmes, and success with any of these approaches will ultimately depend on reprogramming and reorganization of the nuclear architecture of the genome. Contrasting the lower vertebrates, such as zebrafish, which can regrow tissues and recover function, with mammals, which cannot, is currently pursued in an attempt to explain the regenerative mechanisms at a molecular level. This requires a broad, yet deep, understanding of the cross-regulated molecular interactions - the gene regulatory networks (GRNs) – that underscore the success or failure to regenerate.

The fundamental challenge is to decipher how networks of AS events are coordinately regulated to orchestrate adaptive, or conversely maladaptive, responses in diverse biological contexts. At a simplistic level, spatiotemporal specificity of AS is imposed by combinations of trans-acting factors binding to cognate cis-regulatory elements, to direct spliceosome assembly. Although far from trivial in itself, such interactions can be precisely mapped with recently developed technologies such as iCLIP, coupled with high throughput sequencing technologies⁹. However, a complete understanding of the

GRN additionally requires an appreciation of the coordinated interactions with superimposed transcriptional, post-transcriptional, epigenetic and signalling layers of regulation (adding immeasurable complexity). How can we realistically expect to understand, and treat, diseases when most of the pieces of the puzzle are currently “missing”?

As a starting point, a comprehensive register of known splicing regulators is essential to underpin GRN elucidation, but currently lacking. A recent publication by Han, Braunschweig and colleagues¹⁰ describes an innovative technology that can be exploited to define endogenous RNA regulatory networks. Referred to as “Systemic Parallel Analysis of Endogenous RNA Regulation Coupled to Barcode Sequencing” (SPAR-Seq), this quantitative sequencing-based approach enables the systematic discovery of factors that endogenously control AS networks, for example, to impact cell fate. They exemplified the use of the technique by determining networks involved in neural differentiation but the technology could equally be applied to a cardiovascular cell type. A functional genomics screening platform (siRNA knockdown of 1, 536 targets and controls) was combined with a prioritised readout of 52 evolutionarily conserved AS events specifically associated with pluripotency, neural differentiation and direct reprogramming). Multiplex RT-PCR, with barcoding, was followed by high throughput screening which enabled all genes to be assessed for AS changes.

The authors revealed hundreds of novel trans-acting factors that had not previously been implicated in control of splicing, enabling them to elucidate multi-layered mechanisms that influence AS and impact cell fate decisions. SPAR-Seq data were shown to link protein complexes to the control of specific subsets of AS events, providing insight into physical and functional interactions between trans-acting factors and the GRNs that control cell fate. An unexpected observation, in neural cells, was that transcription, chromatin and DNA-binding proteins “multi-task” to impact AS at a similar frequency to splicing factors and that a subset of these proteins dually control cell fate-associated AS networks through both direct and indirect mechanisms. Examples were provided to illustrate the utility of such a screen for elucidating novel mechanisms:

- a previously uncharacterised splicing regulator, *Arglu1*, and a known AS factor, *Srsf2*, displayed opposing patterns of AS; functional validation confirmed the predicted interaction and identified *Arglu1* as a previously unknown splicing regulator that functions by physically antagonising *Srsf2*.
- An example of a transcription factor with dual direct and indirect AS regulatory activities in ES cells was *Nacc1*, found to promote exon inclusion itself and to influence transcription of key AS regulators, including *Mbnl1*.

As well as reporting a valuable methodological advance, this latest study from the Blencowe laboratory at the University of Toronto, reveals novel insights into the orchestration of gene expression. Just as the authors have started to piece together the GRNs governing stem cell multipotency and neural cell differentiation, defining the GRNs that control cardiac and vascular cell fates is clearly feasible. Notwithstanding that the “model” GRNs from such studies need to be formally tested experimentally SPAR-Seq certainly appears to be an efficient resource for generating testable hypotheses for further elaboration of GRNs. Identification of additional important regulators of AS and the delineation of GRNs, layer by layer, with added complexity and crucial insight, will inform the underpinning mechanisms of cardiovascular development and disease and may provide a key to unlock the currently limited regenerative potential of the mammalian heart.

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Biographical Sketch

Dr Nicola Smart is currently a British Heart Foundation (BHF)-funded Senior Research Fellow at the University of Oxford. As a basic scientist, Nicola leads a team of researchers (<https://www.dpag.ox.ac.uk/research/smart-group>) who study the embryonic heart to identify multipotent cardiovascular progenitors and mechanisms that allow the reactivation of developmental processes in the adult for regeneration. Specifically, her work has revealed the potential to reactivate dormant adult epicardial cells to unleash a source of cardiac progenitors for repair of the infarcted heart. The success of Nicola's research has been acknowledged with awards including the inaugural BHF Fellow of the Year and the British Cardiovascular Society's Michael Davies Early Career awards.