

Splice variants' role in mediating different disease states in tissue requiring ion transduction through calcium channel for function

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Abstract

Structure informs function, and this may be the evolutionary reason why alternative splicing, which is capable of generating different variants of the same protein, arise. But, given the energetic cost of generating different splice variants for testing their capability at a specific task, which incurs cellular functional uncertainty; as well as the exertion of differing physiological effects on cells that may translate into diseased states, what is the evolutionary advantage of this process? Additionally, what are the factors that select a specific variant for a presented task? Using heart tissue samples exposed to hypoxia stress as model system, this research idea entails the illumination of single nucleotide polymorphisms (SNP) of the calcium channel transporter, Ca_v 1.2 gene in the population through gene sequencing followed by bioinformatic analysis for alternative splice sites. This would be followed by a scan for alternative splice variants through colony polymerase chain reaction using universal primers for Ca_v 1.2 gene. Confirmation of splice variant identity through Western blot laid the stage for subsequent efforts at cloning and expressing the variant gene in HEK 293 cells lacking endogenous expression of Ca_v 1.2, for biophysical characterization of calcium conduction through patch clamp electrophysiology. In parallel, structural elucidation efforts necessitate the purification of the calcium channel via hydrophobic interaction or reversed phase liquid chromatography after its heterologous expression in a bacterial host. But, biophysical and biochemical characterization does not speak of the signaling and metabolic pathways laying the path to generation of the splice variant(s). Hence, discovery approaches such as RNA-seq and mass spectrometry proteomics could uncover the molecular mysteries at the transcript and protein level that help guide the selection of specific splice variant in response to hypoxic stress, where HIF is a candidate pathway. Implementing this approach from the retrospective angle of examining diseased human tissue samples provide one important facet for uncovering possible mechanisms driving the generation of a splice variant. However, the complementary prospective approach of identifying the molecular basis and processes for responding to hypoxia in a cell line such as HEK 293 would help provide confirmatory evidence in understanding the key drivers of physiological response to lack of oxygen at the cellular level. Collectively, this research route would illuminate both the nucleotide informational basis of alternative splicing in calcium channel Ca_v 1.2 as well as identify the molecular mechanisms enabling the selection of specific splice variants useful for conferring, at the cell and tissue level, ability to withstand hypoxic stress without significant negative effects on cell function. Interested readers can expand on the ideas presented.

Keywords: calcium channel, heart infraction, hypoxia, electrophysiology, alternative splice variant, signaling cascade, transcriptional control, post transcriptional processing, HEK 293 cell line,

Subject areas: genomics, biochemistry, molecular biology, physiology, bioinformatics,

Research idea

Coordinated muscle contraction in the heart require a signaling mechanism for informing individual heart muscle cells to achieve synchronized beating through a calcium wave travelling from calcium channels towards the cytoplasm. Viewed under a confocal microscope with heart cells infused with a fluorescent protein sensitive to calcium, the wave could be seen travelling through an entire sheet of beating cells. But, dysfunction of calcium channels, often manifesting in uncoordinated beating of heart muscle cells, may ultimately lead to heart infraction, where over-exerted cell populations could no longer sustain overall heart function. A transmembrane protein transporter, calcium channels are known to have multitude of splice variants whose functions are not fully understood given their differing roles in different tissues of the heart.

But, a broader question that examine the foundation of calcium channel revolves around the evolutionary selection mechanisms that enabled the generation of a large library of splice variants with different physiological functions. In turn, another interesting question concerns the functions of slightly different splice variants of calcium channels in different tissue contexts such as those in disease, and more importantly, the signaling and metabolic pathway mediating the selection of specific splice sites (and splice variant) over others.

As the second question connects the first, a bioinformatic study would first be used in conducting a broad survey of sequence variation of the Ca_v 1.2 gene in human tissue samples. By aligning sequences and identifying single nucleotide polymorphisms, known splice sites would be confirmed and new ones identified. More importantly, phylogeny and lineages of different families of splice variants could emerge, which lay the basis for understanding the evolutionary forces shaping alternative splicing in the Ca_v 1.2 gene. Away from natural selection forces shaping Ca_v 1.2 nucleotide sequence lies the more important medical question of how different splice variants contribute to a particular disease state alone and in combination. Alternatively, different splice variants could also arise due to onset of cellular dysfunction and help ameliorate disease symptoms. Specifically, while structure function relationship of proteins typically posits a specific protein variant as responsible for particular cellular behaviour, the ensemble of cells in a heart would likely harbor different splice variants of Ca_v 1.2 protein in the same or different cells. To this end, colony polymerase chain reaction utilizing universal primers for Ca_v 1.2 gene would be used to profile different splice variants present in human heart and blood vessel tissue samples. Western blot would subsequently be used for calcium channel identity confirmation as well as providing a semi-quantitative measure of the relative abundance of the splice variant.

Sequencing of the target band from DNA gel electrophoresis provides the nucleotide sequence and identity of splice variant, which enables the cloning and expression of the target splice variant in a cell line without endogenous expression of calcium channel. One such cell line is human embryonic kidney 293 (HEK 293) cell line, which provides an experimental platform for understanding calcium transduction properties of the variant channel through patch clamp electrophysiology studies. Complementary to electrophysiology, structural elucidation of the splice variant provides the structural scaffold for understanding the granular details of calcium movement through the transporter channel. Depending on whether the splice variant yield to crystallization efforts, alternative structural characterization tools such as X-ray free electron laser (XFEL) spectroscopy could also be employed for structure discovery. This, however, requires the expression of the splice variant in microbial chassis and its subsequent purification through a variety of chromatography tools such as hydrophobic interaction chromatography and reversed phase liquid chromatography.

Heretofore, what has been elucidated revolves around the presence and function of the splice variant at the cellular level. But, what are the molecular underpinnings that potentiate the generation of the specific splice variant? Specifically, what are the signal transduction cascade and metabolic processes that yield the splice variant? In heart infraction and stroke patients, lack of oxygen is likely to be a significant factor partaking in cellular physiological processes leading to the generation of specific splice variants. One possible signaling cascade through which hypoxia could mediate its effect is the HIF pathway, which connects extracellular information on oxygen tension to transcription of appropriate effector genes in the nucleus.

Although a candidate pathway important to the transduction of information on oxygen concentration in blood to heart muscle contraction has been hypothesized, this research utilizes discovery science to identify, holistically, all metabolic and signaling pathways important to the generation of a splice variant library for aiding cellular response to hypoxia at the transcriptional and protein level. More importantly, microRNAs and other non-coding RNAs could mediate expression of downstream genes important to an integrated response to hypoxia; thus, RNA-seq would be utilized to profile at the transcript level in tissue samples, all messenger and non-coding RNA present. This information would be augmented with data from mass spectrometric proteomics investigation of cellular extracts of tissue samples from human patients.

Tissues are collection of cells operating to a synchronized tune, and the approach described above comes from the retrospective angle, which provides evidence supporting one of many possible hypothesis concerning the molecular basis of particular clinical manifestations and the mechanisms underlying the selection of a specific splice variant. Thus, forward analysis utilizing HEK 293 cell monolayer in culture subjected to hypoxic stress would help narrow down the candidate molecular effectors important to transducing extracellular stress to selection of a splice variant for coping with cellular dysfunction. RNA-seq and mass spectrometric proteomics would

yield a comprehensive dataset of molecular entities at the transcript and protein levels important to the transduction of hypoxia stress, regulation of splicing, and translation of the candidate splice variant.

Collectively, alternative splicing is not without cost to the cell; thus, natural selection and evolutionary pressure must exist that potentiate the preferential selection of one splice variant over another. But do different splice variants result in differing disease states in tissues? Examining the role of calcium channels in heart disease, where hypoxia (i.e., lack of oxygen) lead to dangerous clinical presentation of cardiac arrest, this research aims to first understand the evolutionary rationale for the generation of different splice variants in calcium channel Ca_v 1.2 gene followed by the correlative analysis of roles of different Ca_v 1.2 splice variants in differing disease states of heart at the human tissue level. Identification of the splice variant at the gene level and confirmation at the protein level would usher into a full investigation on the biophysical (i.e., calcium conduction) and biochemical properties as well as structural biology of the variant using a combination of patch clamp electrophysiology and X-ray diffraction analysis techniques. But, more importantly, the question of what potentiated the generation and selection of specific splice variant would be examined from both the retrospective and prospective angles, where broad based discovery science approaches such as RNA-seq and mass spectrometry enabled proteomics would look for molecular effectors of signal transduction from the extracellular environment to the nucleus beyond the hypothesized HIF pathway, since cross-talks and redundant pathways in biology enable in-built robustness in biological systems.

Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author thought about why evolution imbue genes encoding calcium channels with significant alternative splice sites, which have been utilized by the splicesome to generate a plethora of different incarnations of the same transport protein important to the transduction of calcium into and out of cells in tissue requiring contraction for function. More importantly, given increasing knowledge that these alternative splice variants of calcium channels might be responsible for different disease states, a significant gap in understanding exists in profiling the physiological impact of individual splice variants on calcium channel function in different tissues. The author wrote this manuscript preprint to share his ideas with the scientific community, and interested researchers can expand on them in their research.

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