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Alternative splicing, or differential splicing, is a regulated process during gene expression that results in a single gene coding for multiple calendrierdelascience.com this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene.

Splicing pathways[edit] Several methods of RNA splicing occur in nature; the type of splicing depends on the structure of the spliced intron and the catalysts required for splicing to occur. Introns[edit] The word intron is derived from the terms intragenic region, [1] and intracistron, [2] that is, a segment of DNA that is located between two exons of a gene. The term intron refers to both the DNA sequence within a gene and the corresponding sequence in the unprocessed RNA transcript. As part of the RNA processing pathway, introns are removed by RNA splicing either shortly after or concurrent with transcription. Further upstream from the polypyrimidine tract is the branchpoint, which includes an adenine nucleotide involved in lariat formation. Y-U-R-A-C branch sequence nucleotides upstream of acceptor site This results in a mature messenger RNA with a missing section of an exon. In this way, a point mutation , which might otherwise affect only a single amino acid, can manifest as a deletion or truncation in the final protein. Assembly and activity of the spliceosome occurs during transcription of the pre-mRNA. Two types of spliceosomes have been identified major and minor which contain different snRNPs. By contrast, when the intronic flanking sequences do not follow the GU-AG rule, noncanonical splicing is said to occur see "minor spliceosome" below. However, in some cases, especially in mRNAs with very long introns, splicing happens in steps, with part of an intron removed and then the remaining intron is spliced out in a following step. This has been found first in the Ultrabithorax Ubx gene of the fruit fly, Drosophila melanogaster , and a few other Drosophila genes, but cases in human have been reported as well. Group I and II introns perform splicing similar to the spliceosome without requiring any protein. This similarity suggests that Group I and II introns may be evolutionarily related to the spliceosome. Self-splicing may also be very ancient, and may have existed in an RNA world present before protein. Two transesterifications characterize the mechanism in which group I introns are spliced: The mechanism in which group II introns are spliced two transesterification reaction like group I introns is as follows: The splicing reaction involves a different biochemistry than the spliceosomal and self-splicing pathways. Prokaryotes , on the other hand, splice rarely and mostly non-coding RNAs. Another important difference between these two groups of organisms is that prokaryotes completely lack the spliceosomal pathway. Because spliceosomal introns are not conserved in all species, there is debate concerning when spliceosomal splicing evolved. Two models have been proposed:

Chapter 2 : Alternative splicing - Wikipedia

Spliceosomal introns are just one of the four major classes of introns found in nature, together with group I and group II self-splicing introns, and tRNA introns. Intron types are defined based on various structural and mechanistic features, and they have distinct phylogenetic distributions.

Genes that contain introns are known as discontinuous or split genes as the coding regions are not continuous. Introns are found only in eukaryotic organisms. While it was known that mature eukaryotic mRNA molecules were shorter than the initial transcripts, it was believed that the transcripts were simply trimmed at the ends. When the two molecule types were sequenced it was revealed that this was not the case; much of the removed transcript came from internal regions rather than the extreme ends. This prompted extensive research into how introns were removed from transcripts, and what their role might be. An intron is a stretch of DNA that begins and ends with a specific series of nucleotides. These sequences act as the boundary between introns and exons and are known as splice sites. The recognition of the boundary between coding and non-coding DNA is crucial for the creation of functioning genes. There are other conserved sequences found in introns of both vertebrates and invertebrates including a branch point involved in lariat loop formation. Here we see a consensus sequence for a vertebrate intron. As introns cause an increase in gene length, this increases the likelihood of crossing over and recombination between sister chromosomes. This increases genetic variation and can result in new gene variants through duplications, deletions, and exon shuffling. Introns also allow for alternative splicing. This allows a single gene to encode multiple proteins as the exons can be assembled in multiple ways. As introns are not transcribed, they must then be removed before translation can occur. The excision of introns and the connection of exons into a mature mRNA molecule occurs in the nucleus and is known as splicing. Introns contain a number of sequences that are involved in splicing including spliceosome recognition sites. These sites allow the spliceosome to recognise the boundary between the introns and exons. The sites themselves are recognised by small nucleolar ribonucleoproteins snRNPs. Splicing occurs in three steps: Formation of a lariat or loop structure. This figure shows the splicing of an intron through formation of a lariat. The intron is then removed leaving the two exons connected. It is unknown how the snRNPs and the spliceosome identify which recognition sites to bind to given the that the introns can be thousands of base pairs long and there are many cryptic splice sites where the recognition sequences are found elsewhere in the gene. It is believed that certain proteins for example, SR proteins , enhancers, and silencers are involved. Splicing silencers have also been implicated in human diseases. Alternative splicing Introns and the splicing mechanism also allow for alternative gene products in a process known as alternative splicing. Each discontinuous gene is made up of two or more exons, allowing for multiple ways in which the exons can be assembled. Alternative splicing can result in two to hundreds of different mRNAs. Alternative splicing can occur in a number of ways: Introns in rRNAs are rare, with examples so far found only in lower eukaryotes. Unlike introns in other molecules, some rRNA introns have a unique characteristic â€” they are self-splicing. Self-splicing introns fall into a category known as Group I introns. Rather than relying on an external enzyme to perform the excision the introns themselves act as an enzyme known as a ribozyme. Ribozymes were discovered in the ciliate Tetrahymena in and revolutionized the way scientists viewed enzymes. Introns in tRNAs are relatively short, ranging from 14 to 60 base pairs in length. The introns form part of the stem and loop structure of the tRNA, binding to a section of the anticodon arm. Removal of pre-tRNA introns is done by a single endonuclease. Which organisms do not have introns?

Chapter 3 : RNA splicing - Simple English Wikipedia, the free encyclopedia

Introns, Exons and Splicing hn RNA Introns are a common eukaryotic event. Several features of interrupted genes are: The sequence order is the same as in the mRNA.

Download as PowerPoint Slide Figure 4. Diseases caused by cis-acting mutations that disrupt use of alternative splice sites. Four diseases are caused by mutations that disrupt use of alternative splice sites. The splicing patterns favored by the mutations are indicated by the blue arrows. Red indicates alternatively spliced regions. B Frasier syndrome is caused by mutations in theWT-1 gene. Dotted lines indicate proposed RNA secondary structure. The natural functions of the The other is a deletion that removes both G triplets. Three additional disorders are associated with abnormalities in WT1 expression: All three diseases are characterized by urogenital disorders involving kidney and gonad developmental defects. Consistent with these defects, normal expression patterns of human WT1 during development indicate important roles in kidney and gonad development Armstrong et al. The variable KTS region is located between the third and fourth zinc fingers. The properties of the two WT1 isoforms also indicate that they perform distinct functions. Tau is required for microtubule assembly and function and is thought to play a major role in microtubule-dependent transport in axons. Free tau, not bound to microtubules, is proposed to be subject to hyperphosphorylation and aggregation. MAPT mutations fall into two mechanistic classes. One class includes mutations that alter the biochemical properties of the protein. In vitro analysis of these mutant proteins demonstrated either altered ability to modulate microtubule polymerization or enhanced self-aggregation into filaments that resemble neurofibrillary tangles. A second class of disease-causing mutations that affected splicing was revealed by mutations clustered in and around the alternatively spliced exon 10 Fig. Subsequently, silent mutations in exon 10 were linked to FTDP, ruling out expression of a mutated protein as the pathogenic event Hong et al. Exon 10 encodes the last of four microtubule-binding domains, and exon 10 inclusion is determinative for the ratio of the 4R-tau and 3R-tau protein isoforms 4R and 3R designate four and three microtubule-binding domains, respectively. The vast majority of FTDP mutations affect these regulatory elements and cause disease by increasing inclusion of exon As expected, the 4R-tau protein isoform predominates in the insoluble tau aggregates in individuals with FTDP Hutton et al. This mutation also decreases the 4R-tau protein function in vitro, although the biochemical properties of the recombinant 4R protein are irrelevant if the exon is completely skipped in affected individuals. Unfortunately, the level of exon 10 inclusion in these individuals is unknown because tissue samples are not available. Atypical cystic fibrosis Cystic fibrosis CF is an autosomal recessive disorder caused by loss of function of the cystic fibrosis transmembrane conductance regulator CFTR gene. Fifty percent of affected individuals are homozygous for this allele, resulting in severe pulmonary and pancreatic disease. One is a variant polyuridine tract containing 5, 7, or 9 uridines within the polypyrimidine tract of intron 8. The second is a polymorphic poly UG nlocus immediately upstream of the U n tract. Both polymorphisms are located between the presumptive branch site for intron 8 and the AG-terminal dinucleotide. It is unclear whether this alternative splice serves a purpose. The shortest U n allele, 5U, can be associated with a high level of exon skipping in respiratory epithelial cells compared with the 7U and 9U alleles. This polymorphism is rarely sufficiently penetrant to be associated with a severe CF phenotype Noone et al. On the other hand, the identification of healthy 5Uhomozygotes demonstrated that the penetrance of the 5U allele is quite variable. Variable penetrance is explained in part by the second polymorphic UG n, tract which ranges in size from UG 9 to UG In fact, healthy fathers of individuals affected with CBAVD have been shown to contain shorter UG n polymorphisms and exhibit less exon 9 skipping than their affected sons, explaining the variable penetrance within some families Cuppens et al. Transient transfection analysis ofCFTR minigenes directly demonstrated that the longer UG n tract correlates with increased exon 9 skipping, but only when combined with the 5U allele Niksic et al. The prediction, thus far untested, is that the polypyrimidine tract of the 5U allele binds U2AF65poorly compared with the 7U and 9U alleles and that this interaction is negatively affected by binding of TDP to the upstream UG n tract Buratti et al. Previous Section Next Section Trans effects: Null mutations in spliceosome components are generally lethal or synthetic lethal in yeast and

are most often lethal at the cellular level in metazoans. For example, four components of the basal splicing machinery U2AF35, Sm protein D1, SF3b subunit 4, and U1Ca were identified as genes required for early vertebrate development in a large scale insertional mutagenesis screen in zebrafish Golling et al. All four mutations resulted in nonspecific developmental defects that are thought to result from cell-lethal mutations. Despite the expectation that dysfunction of the basal splicing machinery should be cell-lethal regardless of cell type, mutations that disrupt the assembly or function of spliceosomal snRNPs are responsible for two human diseases in which two different subsets of neurons are affected Fig. Retinitis pigmentosa Retinitis pigmentosa RP is a heterogeneous disease affecting 1 in individuals characterized by progressive retinal degeneration, night blindness, loss of peripheral vision, and ultimately total blindness. The disease results from the specific loss of rod photoreceptor cells. RP can be inherited as an autosomal dominant, autosomal recessive, or X-linked disorder. More than 30 different RP genes and loci have been identified, most of which have retina-specific functions. All three human proteins were found in isolated functional spliceosomes Zhou et al. The mutations include insertions, deletions, missense mutations, and splice-site mutations. It is likely that in at least some mutant alleles, the function of PRPF31 is severely affected, if not completely eliminated. Therefore, PRPF31 mutations are likely to cause autosomal dominant RP due to haploinsufficiency, although a dominant-negative effect for alleles expressing truncated proteins cannot be ruled out. All five examples are caused by one of two missense mutations in two highly conserved adjacent codons in exon This protein domain is unique in the database, and its specific function is unknown. Seven different mutations have been identified in three RP families and four individuals with a history of autosomal dominant RP. All of these mutations cluster in a highly conserved amino-acid region in the last exon. PRP8 is thought to provide overall structural support for the catalytic core and to modulate the RNA helicase activities that control the extensive RNA: RNA base-pairing rearrangements required to activate the spliceosome Collins and Guthrie The remarkable clustering of the mutations identifies a specific functional domain, but it remains to be determined whether these mutations inactivate the allele or create a protein with dominant-negative function. The possible basis for the striking cell-specific effects of the RP mutations is discussed below. Spinal muscular atrophy Spinal muscular atrophy SMA is an autosomal recessive disorder that is one of the most common genetic causes of childhood mortality. The main characteristic of the disease is progressive loss of spinal cord motor neurons, resulting in skeletal muscle denervation with subsequent weakness, atrophy, and paralysis of voluntary muscles. A duplicated gene within the centromeric copy of the inverted repeat SMN2 is also transcribed and contains only a few nucleotide substitutions, none of which alters the protein coding sequence. SMN is a ubiquitously expressed amino-acid protein that is essential in S. The specific functions of SMN are unknown, but it is in a complex Baccon et al. Assembly was restored by adding back purified SMN complex Meister et al. Four clinical types of SMA have been defined based on age of onset and disease severity, which ranges from intrauterine demise to mild symptoms in older individuals. Results from individuals affected with SMA and SMA mouse models demonstrate that there is a clear correlation between SMN protein levels, loss of motor neurons, and disease severity Coovert et al. Unlike humans, mice have only oneSmn gene. This down-regulation also occurs in humans Burlet et al. A muscle-specific knockout of SMN induces severe muscular dystrophy, indicating that substantial reduction of SMN will induce intrinsic muscle disease Cifuentes-Diaz et al. These results indicate that postnatal motor neurons require higher steady-state levels of SMN protein than other metabolically active tissues. For both RP and SMA, the primary defect appears to be a loss of function of essential splicing factors, although dominant-negative function for some RP alleles cannot be ruled out. How can the loss of ubiquitous functions result in such remarkable cell-specific sensitivity? Because exons are diverse units of recognition, different exons are likely to exhibit a wide range of sensitivities to deficiencies of essential splicing factors. It can also be argued that cell-specific pre-mRNAs are more likely to be affected by a deficiency of a basal splicing factor than pre-mRNAs that are widely expressed. In contrast to cell-specific pre-mRNAs, widely expressed pre-mRNAs must have the ability to undergo efficient splicing in a variety of nuclear environments and presumably contain information in cis for more robust splicing. The few essential splicing factors that have been examined in vertebrates show surprisingly variable levels of expression among different tissues that do not correlate with tissue metabolic

activity. For example, SF1, a spliceosome component involved in the initial recognition of the branch site, is barely detectable in pancreas, kidney, and lung, whereas PRP8 is barely detectable in liver Luo et al. Even in yeast, where intron recognition is highly homogenous, loss-of-function phenotypes for PRP2 and CEF1 are due to defective removal of single introns Chen et al. For example, a screen for mutants that disrupt transport of secretory proteins from the endoplasmic reticulum ER to the Golgi identified a well-characterized essential splicing factor, PRP2. Global analysis of splicing in Cef1p mutants using an oligonucleotide array Clark et al. The opsin protein binds covalently to a chromophore to form the photopigment rhodopsin, which undergoes a conformational change in response to photons that initiates the photodetection cascade Bessant et al. Rhodopsin is embedded in the extensive array of membranous discs present in each rod cell. The discs undergo daily renewal just prior to waking Korenbrot and Fernald , putting considerable demand on the splicing machinery to produce huge amounts of opsin mRNA. Analogous potential targets required for motor neuron viability in SMA are less obvious. It is possible that one or only a few pre-mRNAs are affected by the sequence of events resulting from reduced assembly of core snRNPs. All three examples illustrate that inactivation of a splicing regulator in mice specifically affects its natural pre-mRNA targets. The same specificity is expected in human diseases caused by disrupted function of alternative splicing regulators. Myotonic dystrophy Myotonic dystrophy DM is the one human disease in which disease phenotype has been directly linked to disrupted regulation of alternative splicing Fig. DM is an autosomal dominant disorder and the most common form of adult-onset muscular dystrophy, with a worldwide incidence of 1 in DM is unusual because of its phenotypic variability even within families and the diversity of tissues affected. Symptoms include skeletal muscle hyperexcitability myotonia , progressive muscle wasting, cardiac conduction defects, cataracts, smooth muscle dysfunction, testicular atrophy, an unusual form of insulin resistance, and neuropsychiatric and cognitive disturbances Harper Two types of DM have been identified. Disease severity and age of onset correlate with repeat length, which ranges from 80 to thousands of repeats. Several independent lines of evidence indicate that the predominant mechanism for DM pathogenesis is a gain of function for RNA transcribed from the expanded alleles. First, no point mutants or deletions within the DM1 or DM2 loci cause DM, indicating that the repeats are determinative for these diseases rather than a loss of function associated with the DM1 or DM2 loci. Second, the fact that two different loci containing similar expanded repeats cause strikingly similar diseases strongly suggests that DM1 and DM2 share a common pathogenic mechanism that is independent of a loss of function for the affected locus. The IR splicing switch observed in DM1 skeletal muscle results in expression of a lower signaling IR isoform directly correlating with the unusual form of insulin resistance observed in individuals with DM1 Savkur et al.

Chapter 4 : Alternative splicing - Simple English Wikipedia, the free encyclopedia

RNA splicing is the method by which pre-mRNA is made into mature mRNA, by removal of introns and joining together of exons. Several methods of splicing exist, depending on the organism, type of.

Copyright Genetics Society of America Alternative Splicing When molecular biologists began analyzing the complete sequence of the human genome in mid, one surprising observation was that humans have relatively few genes. We may have as few as 30, genes, only about two times as many as the much simpler fruit fly , Drosophila melanogaster. How can the much greater size and complexity of humans be encoded in only twice the number of genes required by a fly? The answer to this paradox is not fully understood, but it appears that humans and other mammals may be more adept than other organisms at encoding many different proteins from each gene. One way they do this is through alternative splicing, the processing of a single RNA transcript to generate more than one type of protein. In most eukaryotic genes, the protein-coding sequences, termed exons, are interrupted by stretches of sequence, termed introns, that have no protein-coding information. After the gene is copied, or transcribed, to RNA, the introns are removed from this "pre-mRNA," and the exons are spliced together to form a mature mRNA , consisting of one contiguous protein-coding sequence. In addition, the complete mRNA contains upstream and downstream sequences flanking the coding sequences. These sequences do not encode protein, but help to regulate translation of the mRNA into protein. Variations in the splice pattern lead to alternative transcripts and alternative proteins. The boundaries between exons and introns in a pre-mRNA are marked very subtly. Certain segments of the pre-mRNA, termed splice sites, direct the spliceosomes to the precise positions in the transcript where they can excise introns and splice together exons. Splice sites are short sequences, typically less than ten bases long. Although splice sites often can be recognized as such by common patterns in their base sequence, there are many variations on the basic splice site consensus sequence. These differences affect how readily a particular splice site is recognized and processed by the splicing machinery. Many other molecules within the cell, called splicing factors, also participate in the splicing reaction. The combination of all of these determines the pattern of splicing for a particular pre-mRNA molecule. For many genes the pattern of splicing is always the same. These genes encode many copies of their corresponding pre-mRNA molecules. The introns are removed in a consistent pattern, producing mature mRNA molecules of identical sequence, all of which encode identical proteins. For other genes the splice pattern varies depending on the tissue in which the gene is expressed, or the stage of development the organism is in. Because the choice of splice sites depends on so many different factors, the same pre-mRNAs from these genes may become spliced into several, or even many, different mature mRNA variants. The production of such mRNA variations through the use of different sets of splice sites is known as alternative splicing. It has been estimated that at least one-third of all human genes are alternatively spliced. Alternative splicing can have profound effects on the structure and function of the protein encoded by a gene. Many proteins are comprised of several domains, or modules, that serve a particular function. For example, one domain may help the protein bind to another protein, while another domain gives the protein enzymatic activity. By alternative splicing, exons, and, therefore, protein domains, can be mixed and matched, altering the nature of the protein. By regulating which splice patterns occur in which tissue types, an organism can fine-tune the action of a single gene so it can perform many different roles. The various forms of a protein are known as isoforms. Isoforms are often tissue-specific. The dystrophin gene, for example, has one form in muscle and another in brain tissue. Defects in alternative splicing are associated with several important human diseases, including amyotrophic lateral sclerosis , dementia, and certain cancers. Alternative splicing can also act to turn genes off or on. In mRNA, codons, consisting of three adjacent nucleotides , either encode an amino acid or signal the ribosome to stop synthesizing a polypeptide. Alternative splicing can introduce a stop codon in the beginning or middle of a protein-coding sequence, resulting in an mRNA that encodes a prematurely truncated polypeptide. Human hearing offers a dramatic illustration of how important alternative splicing is in everyday life. Microscopic hair cells lining the inner ear vibrate when stimulated by sound. One of the proteins in the hair cells that plays a role in the hearing sensation is a calcium-activated potassium

channel. The gene for this protein can generate more than five hundred different mRNA variants through alternative splicing. The resulting potassiumchannel proteins have slightly differing physiological properties. This is in part what tunes hair cells to different frequencies. Muhlrad Bibliography Alberts, Bruce, et al. Molecular Biology of the Cell, 4th ed. An Introduction to Genetic Analysis, 7th ed. Lodish, Harvey, et al. Molecular Cell Biology, 4th ed. The sensitivity of the human ear to a wide range of sound frequencies is due to alternative splicing of a potassium channel gene, giving rise to a set of related proteins whose exact form varies with the position in the cochlea. The protozoan Trypanosome brucei, which causes African sleeping sickness, edits some of its messenger RNA molecules after they are transcribed. Uracil nucleotides are added in some locations in the mature RNA and deleted from others. Similar cases of RNA editing occur in other organisms, and even in humans. The human apolipoprotein B gene is edited in the intestine but not in the liver, leading to two distinct forms of the protein, serving different functions in the two organs. Cite this article Pick a style below, and copy the text for your bibliography.

Chapter 5 : gene expression - Can intron become exon in alternative splicing? - Biology Stack Exchange

However, in eukaryotes, before the mRNA can be translated into proteins, non-coding portions of the sequence, called introns, must be removed and protein-coding parts, called exons, joined by RNA splicing to produce a mature mRNA.

It is also called alternative RNA splicing. In alternative splicing, interactions between different proteins, the cell, and the environment can cause different segments of the original DNA to be omitted from the mRNA. When this happens, the alternate mRNA is translated into an entirely different protein. Proteins differ only in the basic arrangement of their amino acids, which is dictated by the mRNA. Once that is changed, the function of the protein changes. Using the method of alternative splicing, organisms can produce many more proteins than their DNA might indicate. For instance, humans have around 20, genes which code for a protein. However, there are thought to be over , different proteins in the human body. Alternative splicing creates these different forms. How Does Alternative Splicing Work? They both rely on 4 nucleotide bases. When a ribosome reads this language, it translates the message into the language of proteins, which consists of around 21 amino acids. Therefore, before a primary mRNA is translated into a protein, it must first be modified and edited. The primary mRNA has various regions, called introns and exons. These regions are mixed together, and the introns must be removed to create a functional protein. The spliceosome is specially equipped to remove the introns. These special strands of RNA contain sequences of nucleotides which match specific locations in the exons and bind to them. The protein portion of the spliceosome then acts as an enzyme, removing the introns and binding the exons together. This spliced mRNA is now ready to be translated into a protein. However, alternate splicing can also take place. While the entire mechanism is not well understood, it is known that certain chemical factors can stimulate the spliceosome to operate in different ways. A signal may be given to exclude an exon, or even multiple exons from the final mRNA. Other signals and pathways can cause the spliceosome to leave introns intact or skip large sections of the protein. Our bodies have many different uses for proteins, and can often use the same DNA blueprint to make many of these proteins. See the examples section for specific examples. Below is a generalize chart showing the different ways a spliceosome can alternatively splice a primary RNA. There is another form of alternative splicing, known as trans splicing, in which exons from two different genes get assembled together by a spliceosome. This genetic process has only been observed in a few single-celled organisms, but could help explain their genetic diversity without sexual reproduction. While sexual reproducing organisms must breed to mix their genetics and produce new varieties, these organisms can do it much faster. This form of alternative splicing can easily create entirely new functions in these organisms, which may prove to be beneficial. Examples of Alternative Splicing Neurexin Genes Humans have 3 genes which code for a family of proteins known as neurexins. These proteins are incorporated into the plasma membrane. They extend out of the plasma membrane and into the space between nerves. Here, they bind to a protein from the other nerve cell. This protein complex holds the cells in place. While there are only 3 different genes which code for neurexins, there are over 3, different proteins in the neurexin family. This is possible through alternative splicing. As the spliceosome processes the primary mRNA molecules from these genes, it is influenced by a number of promoter genes, molecules in the cell, and other signals. These influence which exons get included into the final mRNA. The alternate splicing can make the proteins larger or smaller, or with regions missing, but it generally still produces a working protein. In this way, each variation of cellular environment or extracellular signal creates a different protein with a slightly different function. While all the neurexin proteins will function in holding together the synapse between two nerves, the variation produced is theorized to do a number of things. First, it may alter the signal traveling between the two neurons. This could produce a necessary effect for the brain to process the signal. Different proteins may be employed at different times, in different cells, in the same animal. This might be necessary to accommodate the many different environments within an organism, and ensure the neurons are working properly. When scientist observed the same genes in fish, they found something interesting. While fish also have these genes, they cannot splice the genes into nearly as many alternatives. This lead scientists to hypothesize that alternative splicing might be used to modify these genes in a way which makes them specific

to certain parts of the brain. This might be the reason humans can store so much extra information and has such efficient long term memory. Making Antibodies In a similar process, the human body makes antibodies to fight bacteria, viruses, and foreign bodies which infect the tissues. To do this, the body must make an antibody, or protein which is specifically designed to stick to the invader. These proteins are manufactured by B lymphocytes, which contain the DNA and machinery to create these complex proteins. However, there is a problem. The B lymphocytes need to attach the protein to themselves, and they need to release the antibody into the bloodstream. The antibody in the bloodstream will bind to invaders, allowing immune cells to target them. By attaching antibodies directly to the B lymphocytes, these cells can easily swallow up the invaders as they encounter them. To do this with minimal energy and by using the same DNA, these immune cells use alternative splicing. The last two exons in the genetic code for antibodies are special. These two exons encode for a region of protein which is hydrophobic, or resists water. These regions attach themselves within the hydrophobic core of the phospholipid bilayer. This effectively locks them into the cell membrane. Alternative splicing simply removes these two exons. Now, the protein will serve the same purpose, but it is water soluble and can travel through the blood and fluids. Upon receiving a signal to create antibodies, the B lymphocyte must create many at once for both itself and to be release into the body. To do this, it actively transcribes the gene for the antibody quickly, to create as many primary transcripts as possible. Some of these will be processed to retain the hydrophobic region, and some spliceosomes will cut that out. Thus, proteins for both uses are created from the same signal to create antibodies. Alternative splicing makes it possible to initiate many different processes from the same DNA transcription signal. What is the main purpose of alternative splicing? To create variants of proteins B. To assist with the metabolism C. To speed the process of creating proteins Answer to Question 1 A is correct. Alternative splicing allows organisms to store the information for an entire family of genes in the same place. Because the genes can be edited, spliced differently, and modified, they can create many more actual proteins than number of genes they have. Why do organisms need so many versions of the same protein? For the thousands of different functions their cells complete C. Scientists have yet to fully determine the function of alternative splicing. All of the above may be correct, or none of them. It does seem to be related to increased complexity. However, the common fruit fly is one of the organism with the most complex alternative splicing schemes we have studied. How might alternative splicing help create intelligence? By producing different proteins, it can create advanced neural connections B. The more protein you have, the smarter you are C. It is unlikely alternative splicing creates intelligence Answer to Question 3 A is correct. Alternative splicing can essentially allow the brain to map the connections between different nerves, and designate specific nerves for certain tasks. This is the basis of intelligence and memory. The more specialized connections the brain has, the more an organism can remember and process. From Genes to Genomes.

Chapter 6 : Pre-mRNA splicing and human disease

Alternative splicing. Alternative splicing (AS) is a process by which exons can be either excluded or included in or from a pre-mRNA resulting in multiple mRNA isoforms.

These two classes of ribozymes are distinguished by their mechanisms of splicing and by their unique structures 1. Reaction Mechanisms Both classes of self-splicing introns perform two consecutive transesterification reactions in the process of exon ligation. The second step is analogous to the reverse of the first step. Both the first and second steps of the splicing reaction are fully reversible because no net energy is consumed. Two-step reaction mechanisms for the self-splicing group I and group II classes of introns. Unlike the group I introns, a group II intron utilizes an internal nucleophile for the first step of splicing reviewed in Ref. This results in ligation of the flanking exons and release of the lariat or linear intron. The reaction catalyzed by the group II intron follows the same mechanism as that employed in the more complex process of messenger RNA precursor splicing catalyzed by the spliceosome 4. For this reason, it has been proposed that group II intron splicing is an evolutionary precursor to pre-mRNA splicing. Structures Group I and group II introns catalyze their self-splicing reactions by folding into a distinct tertiary structures comprised of many conserved secondary structural elements 5, 6. The hallmarks of a group I intron include a common secondary structure of 10 paired segments termed P1-P10 Fig. The "catalytic core" of the intron is made up of about nucleotides that are highly conserved among group I introns isolated from a broad diversity of biological sources. The double-headed arrows indicate a few sites of tertiary interaction between different regions of the intron. An excellent example of this structural convergence was observed within the P4-P6 domain of the Tetrahymena group I intron 7. The crystal structure of this independently folding domain of the intron was recently reported 8. The structure is formed from two long helical segments helices P6, P4, P5 and helices P5b, P5a that pack side by side. Several new motifs of RNA structure and for metal binding were identified within this folding domain, including the "A-platform" and the "ribose zipper" 8, 9. It comprises about half of the intron active site but does not include the P1 helix or the guanosine binding site. Models have been proposed for the complete structures of four different group I introns. Although there are substantially fewer conserved nucleotides, a group II intron also has a distinct structure 6 Fig. The intron is organized into six double-helical domains D1-D6 that originate from a central wheel. Each of the domains has a particular function in the activity of the intron, although D1, D5, and D6 form the intron core. Interdomain interactions also allow domain I to serve as the scaffold upon which the intron active site is built. In contrast to domain 1, domain 5 is a relatively small nucleotide helical element, yet a large percentage of the phylogenetically conserved nucleotides is concentrated in this domain. It constitutes the catalytic center of the group II intron. Schematic secondary structure of the group II intron. The bulged A, which acts as the nucleophile in the first step of splicing, is circled in Domain 6. Arrows represent the splice sites. They are extremely widespread across phylogeny and have been found in mitochondrial, chloroplast, and nuclear genomes of diverse eukaryotes, although they have not yet been observed in vertebrates. The discovery of a group I intron in T4 bacteriophage was the first example of RNA splicing observed in a prokaryote. Group I introns have also been found in eubacterial genomes. There are currently more than examples of group I introns in the genomic databases. All known group II introns are located within eukaryotic organelles, including plant and fungal mitochondrial DNA and the majority of introns in plant chloroplasts 1. Metals in Folding and Catalysis Both group I and group II introns are metalloenzymes, which require divalent metal cations for activity Although the metal specificity varies substantially among group I introns, it is usually satisfied by Mg or Mn. Group II splicing is substantially less efficient and requires nonphysiological concentrations of monovalent and divalent cations as high as 2. Three Mg ions coordinate to separate phosphate groups within the subdomain This allows the RNA to fold inside-out, that is, the phosphates point into the structure and the nucleotide bases point out to the solvent. Some structural metals in RNA can often be substituted with polycations, such as spermidine or cobalt hexamine, which emphasizes the importance of charge neutralization in RNA folding. Biochemical evidence has implicated two metal ions in the chemical transition state of group I intron splicing

14, One of these metals activates the nucleophile, and the second stabilizes the leaving group during the transesterification reaction. These metal-binding sites are highly selective and cannot be substituted with a generic polycation. A two-metal active site has also been proposed for the group II intron reaction mechanism, although the evidence for this mechanism is not as complete A ribozyme active site that has two metal ions is analogous to those seen in protein polymerases that catalyze the transesterification reactions of replication and transcription Accessory Protein Factors Although some group I and group II introns undergo efficient self-splicing in vitro, several have no in vitro splicing activity. This is true of the majority of the group II introns that have been isolated. Splicing of these introns is likely to be promoted by accessory protein factors that assist the RNA in forming the appropriate active structure. Even the yeast mitochondrial group II introns that have in vitro splicing activity require nuclear genes to splice efficiently in vivo Multiple-Turnover Catalysis Both the group I and group II introns can be converted into ribozymes capable of multiple-turnover catalysis. In this form, the ribozyme is not covalently altered during the reaction, and the RNA can cleave multiple substrates. Analysis of this construct demonstrated that the chemical step is slower than the association or dissociation rates for substrate binding

Chapter 7 : Intron - Definition, Function and Structure | Biology Dictionary

Melissa Moore talks about RNA processing to remove non-coding sequences, alternative splicing to produce more than one protein from a single gene, and the spliceosome.

By Yevgeniy Grigoryev Genetic information of an organism is stored in the genes, the functional subunits of the genome, arranged in the strands of the DNA double helix in the nucleus. However, in eukaryotes, before the mRNA can be translated into proteins, non-coding portions of the sequence, called introns, must be removed and protein-coding parts, called exons, joined by RNA splicing to produce a mature mRNA. The discovery of alternative splicing RNA splicing was first discovered in s in viruses and subsequently in eukaryotes. Not long after, scientists discovered alternative patterns of pre-mRNA splicing that produced different mature mRNAs containing various combinations of exons from a single precursor mRNA. The first example of alternative splicing of a cellular gene in eukaryotes was identified in the IgM gene, a member of the immunoglobulin superfamily. Alternative splicing AS therefore is a process by which exons or portions of exons or noncoding regions within a pre-mRNA transcript are differentially joined or skipped, resulting in multiple protein isoforms being encoded by a single gene. This mechanism increases the informational diversity and functional capacity of a gene during post-transcriptional processing and provides an opportunity for gene regulation Figure 1. Alternative Splicing generates transcriptome diversity and provides an opportunity for gene regulation. Alternative splicing can generate mRNAs encoding proteins with different, even opposite functions. Figure used by permission. The mechanism of alternative splicing During alternative splicing, cis-acting regulatory elements in the mRNA sequence determine which exons are retained and which exons are spliced out. These cis-acting regulatory elements alter splicing by binding different trans-acting protein factors, such as SR Serine-Arginine rich proteins that function as splicing facilitators, and heterogeneous nuclear ribonucleoproteins hnRNPs that suppress splicing. Inhibition of silencing could be achieved sterically, when binding of splicing inhibitors to splicing silencers located in close proximity to splicing enhancers blocks the binding of snRNPs and other activator proteins or prevents the spliceosome assembly. The final decision to include or splice an alternative exon is thus determined by combinatorial effects, cellular abundance, and competitive binding between SR activators and hnRNP inhibitors. The outcome of alternative splicing depends on the stoichiometry and interactions of splicing activators and inhibitors as well as the steric conformation and accessibility of the splicing sites. Recent advances in high-throughput technologies have facilitated studies of genome-wide alternative splicing. Furthermore, there is evidence for alternatively splicing events that are often differentially regulated across tissues and during development, as well as among individuals and populations, suggesting that individual isoforms may serve specific spatial or temporal roles. Alternative splicing is known to be involved in the regulation of normal physiological functions as well as pathologies. For example, a number of alternatively spliced genes in immunity are known. Studies indicate that alternative splicing of CD44, a protein involved in T cell homing with 10 variable cassette exons and six distinct protein isoforms, is crucial for T cell function. The variable exons of CD44 all encode portions of the membrane-proximal extracellular domain of the protein, and the presence of some of the variable exons has been shown to increase the association of CD44 with various proteins. Isoform expression has been shown to be activation dependent, such that the naive T cells mainly express the smallest CD44 isoform that lacks all variable exons, whereas activated T cells express multiple CD44 isoforms, indicating that CD44 alternative splicing is important for activation. While these examples demonstrate the utility of alternative splicing in humans, the scope and exact role of this regulatory mechanism still remains to be investigated on a genome-wide scale. Current technological advances suggest that alternative splicing is more widespread than initially thought and is likely to be involved in a number of human pathologies.

Chapter 8 : Transcription: Introns, Exons and Splicing hn RNA

Alternative Splicing, Exons, Introns, Post-Transcriptional Modifications, RNA Splicing What is RNA Splicing RNA splicing is the biological process that removes the introns from the primary RNA transcript while ligating the exons together in eukaryotes.

Introns are a common eukaryotic event. Several features of interrupted genes are: The sequence order is the same as in the mRNA The structure of an interrupted gene is identical in all tissues. Introns of nuclear genes have termination codons in all three reading frames. Eubacteria are the only species in which introns have not been found. In general, genes that are related by evolution have related organizations with conservation of the position at least some introns. Furthermore, conservation of introns is also detected between genes in related species. The amount and size of introns varies greatly. The mammalian DHFR has 6 exons that total about bases, yet the gene is 31, bases. Likewise, the alpha-collagen has 50 exons that range from bases and the gene is about 40, bases. Clearly two genes of the same size can have different number of introns, and introns that vary in size. Some species will have an intron in a gene, but another species may not have an intron in the same gene. An example is the cytochrome oxidase subunit II gene of plant mitochondria where some plant species have an intron in this gene and others do not. Features of Nuclear Splicing Junctions No extensive homology exists between the ends of an intron. This sequence is about 30 bases upstream of the right exon junction. This produces a lariat structure. The right junction is cut, the lariat becomes single-stranded and is degraded, and the exons are spliced. Splicing appears to involve a complex called the spliceosome. This complex consists of RNA and protein, and appears to be composed of a group of small nuclear ribonucleoprotein particles or snRNPs. These snRNPs each seem to have a role in the splicing process. We will talk about just one snRNP, U1. An important question is whether U1 is required. If the sequence of the splice site is mutated, binding to the left junction will not occur. But if the U1 RNA is altered to be complimentary to the mutation in the left junction, binding is restored. The following is a summary table.

Chapter 9 : Origin of spliceosomal introns and alternative splicing.

Unlike the group I introns, a group II intron utilizes an internal nucleophile for the first step of splicing (reviewed in Ref. 3) (Fig. 1). The 2â€²-OH of a highly conserved bulged A nucleotide located within domain 6 of the intron attacks the 5â€²-splice site.

In nematodes , the mean is 4â€”5 exons and introns; in the fruit fly Drosophila there can be more than introns and exons in one transcribed pre-mRNA. The exons to be retained in the mRNA are determined during the splicing process. The regulation and selection of splice sites are done by trans-acting splicing activator and splicing repressor proteins as well as cis-acting elements within the pre-mRNA itself such as exonic splicing enhancers and exonic splicing silencers. The typical eukaryotic nuclear intron has consensus sequences defining important regions. The nucleotide at the branchpoint is always an A; the consensus around this sequence varies somewhat. In humans the branch site consensus sequence is yUnAy. The complex at this stage is known as the spliceosome A complex. Formation of the A complex is usually the key step in determining the ends of the intron to be spliced out, and defining the ends of the exon to be retained. U1 and U4 leave. The remaining complex then performs two transesterification reactions. The intron is then released in lariat form and degraded. However, as part of the complexity of alternative splicing, it is noted that the effects of a splicing factor are frequently position-dependent. That is, a splicing factor that serves as a splicing activator when bound to an intronic enhancer element may serve as a repressor when bound to its splicing element in the context of an exon, and vice versa. Splicing silencers are sites to which splicing repressor proteins bind, reducing the probability that a nearby site will be used as a splice junction. These can be located in the intron itself intronic splicing silencers, ISS or in a neighboring exon exonic splicing silencers , ESS. They vary in sequence, as well as in the types of proteins that bind to them. The context within which regulatory elements act includes cis-acting context that is established by the presence of other RNA sequence features, and trans-acting context that is established by cellular conditions. For example, some cis-acting RNA sequence elements influence splicing only if multiple elements are present in the same region so as to establish context. As another example, a cis-acting element can have opposite effects on splicing, depending on which proteins are expressed in the cell e. The adaptive significance of splicing silencers and enhancers is attested by studies showing that there is strong selection in human genes against mutations that produce new silencers or disrupt existing enhancers. In males, exons 1,2,3,5,and 6 are joined to form the mRNA, which encodes a transcriptional regulatory protein required for male development. In females, exons 1,2,3, and 4 are joined, and a polyadenylation signal in exon 4 causes cleavage of the mRNA at that point. The resulting mRNA is a transcriptional regulatory protein required for female development. Females, however, produce the splicing activator Transformer Tra see below. The SR protein Tra2 is produced in both sexes and binds to an ESE in exon 4; if Tra is present, it binds to Tra2 and, along with another SR protein, forms a complex that assists U2AF proteins in binding to the weak polypyrimidine tract. U2 is recruited to the associated branchpoint, and this leads to inclusion of exon 4 in the mRNA. Drosophila Transformer[edit] Alternative splicing of the Drosophila Transformer gene product. Pre-mRNAs of the Transformer Tra gene of Drosophila melanogaster undergo alternative splicing via the alternative acceptor site mode. The gene Tra encodes a protein that is expressed only in females. The primary transcript of this gene contains an intron with two possible acceptor sites. In males, the upstream acceptor site is used. This causes a longer version of exon 2 to be included in the processed transcript, including an early stop codon. The resulting mRNA encodes a truncated protein product that is inactive. Females produce the master sex determination protein Sex lethal Sxl. This prevents the use of this junction, shifting the spliceosome binding to the downstream acceptor site. Splicing at this point bypasses the stop codon, which is excised as part of the intron. The resulting mRNA encodes an active Tra protein, which itself is a regulator of alternative splicing of other sex-related genes see dsx above. Fas receptor[edit] Alternative splicing of the Fas receptor pre-mRNA Multiple isoforms of the Fas receptor protein are produced by alternative splicing. Two normally occurring isoforms in humans are produced by an exon-skipping mechanism. An mRNA including exon 6 encodes the membrane-bound form of the Fas receptor, which

promotes apoptosis , or programmed cell death. Increased expression of Fas receptor in skin cells chronically exposed to the sun, and absence of expression in skin cancer cells, suggests that this mechanism may be important in elimination of pre-cancerous cells in humans. The inclusion or skipping of the exon depends on two antagonistic proteins, TIA-1 and polypyrimidine tract-binding protein PTB. If U1 does not bind, the exon is skipped see "a" in accompanying figure. This mechanism is an example of exon definition in splicing. However, recently studied examples such as this one show that there are also interactions between the ends of the exon. In this particular case, these exon definition interactions are necessary to allow the binding of core splicing factors prior to assembly of the spliceosomes on the two flanking introns. Competition between the activator and repressor ensures that both mRNA types with and without exon 2 are produced. It might be more correct now to say "One gene â€" many polypeptides". Since the methods of regulation are inherited, this provides novel ways for mutations to affect gene expression. Several proteins can be encoded by a single gene, rather than requiring a separate gene for each, and thus allowing a more varied proteome from a genome of limited size. A single point mutation may cause a given exon to be occasionally excluded or included from a transcript during splicing, allowing production of a new protein isoform without loss of the original protein. Such functional diversity achieved by isoforms is reflected by their expression patterns and can be predicted by machine learning approaches. This finding led to speculation that the perceived greater complexity of humans, or vertebrates generally, might be due to higher rates of alternative splicing in humans than are found in invertebrates. When they compared alternative splicing frequencies in random subsets of genes from each organism, the authors concluded that vertebrates do have higher rates of alternative splicing than invertebrates. Abnormally spliced mRNAs are also found in a high proportion of cancerous cells. For certain types of cancer, like in colorectal and prostate, the number of splicing errors per cancer has been shown to vary greatly between individual cancers, a phenomenon referred to as transcriptome instability. Mutation of DNMT3A has been demonstrated to contribute to hematologic malignancies , and that DNMT3A -mutated cell lines exhibit transcriptome instability as compared to their isogenic wildtype counterparts. In two separate studies, expression of two of these abnormally spliced mRNAs in mammalian cells caused changes in the DNA methylation patterns in those cells. Cells with one of the abnormal mRNAs also grew twice as fast as control cells, indicating a direct contribution to tumor development by this product. An important property of cancerous cells is their ability to move and invade normal tissue. The abnormal isoform of the Ron protein encoded by this mRNA leads to cell motility. These insights suggest that epigenetic regulation determines not only what parts of the genome are expressed but also how they are spliced. Typically, alternatively spliced transcripts have been found by comparing EST sequences, but this requires sequencing of very large numbers of ESTs. Most EST libraries come from a very limited number of tissues, so tissue-specific splice variants are likely to be missed in any case. High-throughput approaches to investigate splicing have, however, been developed, such as: These methods can be used to screen for polymorphisms or mutations in or around splicing elements that affect protein binding. When combined with splicing assays, including in vivo reporter gene assays, the functional effects of polymorphisms or mutations on the splicing of pre-mRNA transcripts can then be analyzed. The array is then probed with labeled cDNA from tissues of interest. This can reveal the presence of particular alternatively spliced mRNAs. A trans-acting splicing regulatory protein of interest is then precipitated using specific antibodies. When the RNA attached to that protein is isolated and cloned, it reveals the target sequences for that protein. This method has been used to isolate mutants affecting splicing and thus to identify novel splicing regulatory proteins inactivated in those mutants. These databases are useful for finding genes having pre-mRNAs undergoing alternative splicing and alternative splicing events.