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Mini Review

Human intronless genes: Functional groups, associated diseases, evolution, and mRNA processing in absence of splicing

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ABSTRACT

Intronless genes (IGs) constitute approximately 3% of the human genome. Human IGs are essentially different in evolution and functionality from the IGs of unicellular eukaryotes, which represent the majority in their genomes. Functional analysis of IGs has revealed a massive over-representation of signal transduction genes and genes encoding regulatory proteins important for growth, proliferation, and development. IGs also often display tissue-specific expression, usually in the nervous system and testis. These characteristics translate into IG-associated diseases, mainly neuropathies, developmental disorders, and cancer. IGs represent recent additions to the genome, created mostly by retroposition of processed mRNAs with retained functionality. Processing, nuclear export, and translation of these mRNAs should be hampered dramatically by the lack of splice factors, which normally tightly cover mature transcripts and govern their fate. However, natural IGs manage to maintain satisfactory expression levels. Different mechanisms by which IGs solve the problem of mRNA processing and nuclear export are discussed here, along with their possible impact on reporter studies.

1. Why do we have introns?

Intron-containing genes are common in higher eukaryotes and comprise most of the genes in the human genome. Despite the obvious disadvantages of having introns, including energy loss and slower protein production, genomes of the higher (or maybe call them more complex?) eukaryotes are packed with introns, suggesting that exon–intron gene structure provides an enormous advantage, at least for the complex multicellular organisms. The number of non-protein-coding insertions in the genome (i.e. introns and intragenic sequences) has been shown to decrease with population size [1]; therefore, unicellular, high breeding eukaryotes, such as yeast, have a very small share of introns, whereas mammalian genomes are dominated by them. Introns are especially prevalent in the human genome. Humans have the highest average number of introns per gene (8–9) [2]. The gene encoding the giant muscle protein titin has a record-breaking 363 exons [3]. Introns comprise 24% of the human genome, compared to the mere 1.1% comprised of exons. The largest human introns reach approximately 1 Mb (e.g., *KCNIP4* or *ACCN1*, which encode cation channel proteins). The functionality of long introns remains unknown, but we can contemplate an astonishing evolutionary experiment: the genome of the puffer fish *Takifugu rubripes* (and ~25 related species) has shrunk considerably, leaving short introns of approximately 60–150 nt [4]. Although a vast portion of its genomic material has vanished, the fish's phenotype does not seem to have been affected, which underlines the question of introns functionality. Several answers to this question have been developed:

- The intron–exon gene structure enables alternative splicing, which generates diversity in protein products derived from a single gene (more than 90% of human genes are alternatively spliced).
- Efficient splicing enables cellular machinery to distinguish between correct coding messengers and incorrect faulty messengers. Introns are essential for nonsense-mediated decay (NMD) in which a premature stop codon positioned too close to the exon–junction complex triggers mRNA degradation. This mechanism is responsible not only for the elimination of incorrect mRNAs, but also the regulation of some perfectly normal messengers, such as mRNA encoding neuronal plasticity protein Arc [5]. In addition, a number of transcripts in the mammalian genome are much larger than previously expected and much larger than the number of protein-coding genes [6]. Although recent reports have revealed that the abundance of this RNA "dark matter" may have been overestimated [7], random arbitrary transcription still likely generates a portion of non-functional transcripts in the human genome. Splicing may serve as a tool for the separation of functional mRNAs from random transcripts because splicing factors bind to mRNA during the process and assemble complexes important for proper mRNA maturation, nuclear export, and the regulation of translation, ensuring efficient expression.

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- Introns may contain non-coding, regulatory sequences [8–10], non-coding RNAs (all snoRNAs, many microRNAs, and long non-coding RNAs), or "nested genes" encoding some other proteins, usually intronless ORFs (e.g., vesicular acetylcholine transporter (VChAT) encoded in the first intron of the choline acetyltransferase (ChAT) gene) [11]. This last example is also a good illustration of the co-regulation of two genes engaged in the same process.

Thus, the presence of introns seems to be important for the precise fine-tuning of gene expression, and especially important in organisms with complex embryogenesis, neuronal development, or immune responses. Aware of this context, we may reflect on the origin, functions, and processing of human intronless genes.

2. Intronless genes database

The Human Intronless Genes Database (IGD) created and described by Louhichi et al. [12] is currently the only online database assembling intronless genes (IGs) exclusively. This database is curated and inappropriate entries, such as multi-exonic genes, redundant hits, pseudogenes, or non-protein coding sequences, have been eliminated. Accordingly, from the initial 3477 putative IGs only 687 entries remained, from which only 323 genes can be functionally analyzed. Examination of the chromosomal distribution revealed that, for most chromosomes, the fraction of IGs is close to the fraction of total genes, with the exception of chromosomes 5, 6, 11, and X in which IGs are over-represented, and chromosome 1 in which they are strongly under-represented [12].

3. Functional classification of intronless genes

IGs, although diverse, encode mostly for the receptors, signaling and regulatory molecules important in growth and proliferation, with a relatively small proportion of metabolic enzymes [12]. Histone-encoding genes comprise approximately 20% of IGs, but they represent an exception in every aspect – evolutionarily, functionally, and in terms of mRNA processing. Histones are basic nuclear proteins responsible for the nucleosome structure and for determining centromere formation and centrosome position. Notably, some non-histone IGs encode proteins involved in the determination of centrosome position and segregation (CENPB, CETN1) and in nucleosome assembly (NAP1L2, 3, 5). Whether these genes are in some way co-regulated with histone mRNAs, presumably at the level of mRNA processing, would be interesting to investigate.

The biggest functional group among IGs includes genes encoding proteins with signal transduction activity. These genes represent roughly 53% of all entries, and most of them belong to a group of G-protein-coupled receptors (GPCRs). Approximately 50% of GPCRs are intronless [13]. GPCRs regulate essential physiological functions, controlling neurotransmission, endocrine signaling, immune responses, muscle contraction, blood pressure, and sensory functions. Thus, it is not surprising that they comprise about 50–60% of all current therapeutic targets [14]. GPCRs share a common structural motif with seven membrane-spanning domains. After activation by ligand binding, GPCRs undergo a conformational change and promote the exchange of GDP/GTP, which leads to stimulation of the downstream effectors.

IGs encoding GPCRs include genes for adrenergic receptors (ADRA2 A, B, C, ADRA B1, 2), melanocortin receptors (MC1R, MC3R, MC4R), angiotensin receptors (oncogene MAS1 and MAS-related genes), frizzled receptors involved in Wnt signaling, controlling cell proliferation and polarity (FZD1, 2, 7, 8, 10), and many olfactory and taste receptors.

Although genes encoding histones and GPCRs constitute the largest two groups of IGs, the rest can also be categorized into some interrelated subsets:

- Genes encoding transcription factors, serine/threonine kinases, and signal transduction proteins involved in the regulation of growth and proliferation, including proto-oncogenes (JUNC, JUNB, JUND, Ras-related ERAS, RHOB – RhoA-like, RAP2B, RRAG-A, MOS, MAFA and B, MAGE family members, TOB1 anti-proliferative factor, SFN 14–3-3 sigma signal transduction protein).
- Genes involved in the regulation of development, e.g., forkhead family (FOX genes), involved in the regulation of embryogenesis, cell cycle and tumorigenesis; the SOX–SRY-related HMG-box family of transcription factors involved in the regulation of embryonic development; genes involved in creating morphogenic gradients (NANOS 1, 2, 3, NOG–noggin); genes regulating neuronal development (e.g., NDNL2, CLDN4, MAB21-like, and POU3F2).
- Genes with testis-specific expression, which often participate in sperm formation (e.g., CCIN, necessary for normal morphology during sperm differentiation; CAPZA3, important in determining sperm architecture and male fertility; MBD3L1, active in the postmeiotic stages of sperm development; PGK2, the testis-specific phosphoglycerate kinase; SRY, the testis-determining factor that initiates male sex determination; TCEB3B, C, the testis-specific transcription elongation factor complex; and TSPYL, a testis-specific nucleosome assembly protein).
- Genes encoding proteins of the immune system (15 interferon-coding genes, including INFα-1 and INFβ-1).

There are also small subsets of other genes among IGs, encoding molecular chaperones, (including HSPA1A and B, HSPA6: members of the heat shock 70 family, DNAJC30, DNAJB7: members of the heat shock 40 family, HSPB3, and HSPB9), potassium channels (KCNA5, 6, KCNE1L, KCNF1, KCNJ11, KCTD11, 12) and procadherins (PCDHB1-16).

Overall, the functional distribution of IGs is significantly different from the distribution observed in the whole set of human genes, with a predominance of regulatory, signal-transducing molecules responsible for the regulation of growth, proliferation, development, sperm formation, and immune responses.

4. Diseases associated with intronless genes

Intriguingly, an unusually high proportion of IGs are testis or neuro-specific. The functional bias and tissue-specific expression translate into types of diseases associated with an abnormal expression of IGs, including neurological or developmental disorders, neuropathies, eye-malformations, infertility-associated syndromes, and several types of cancer, especially leukemias (Table 1).

5. Evolution of IGs

Evolutionarily, human IGs emerged relatively recently [15], with remarkably conserved core-histones representing a significant exception. These genes are often intronless copies of existing genes, with different specificity or functionality. The most plausible explanation for IGs is retroposition of cellular mRNAs by retrotransposable elements littering eukaryotic genomes [16]. In humans, retro-elements comprise approximately 40% of the genomic material [16,17]. Many of the young human IGs emerged after a burst of retroposition in primates approximately 38–50 million years ago [18]. The L1 retroposon in the human genome belongs to the family of long interspersed elements (LINEs) and provides two activities crucial for retroposition: endonuclease and reverse

Table 1
Diseases associated with intronless genes.

Diseases	Genes
Neurodegeneration/neurological problems	
Williams–Beuren syndrome, a neurodevelopmental disorder	CLDN4
Depression	DCNP1
Adolescent progressive myoclonus epilepsy (Lafora disease)	EPM2A
	NHLRC1
Distal hereditary motor neuropathy type 2C	HSPB3
Cardiac and neurological abnormalities found in the AMME (contiguous gene syndrome, Alport syndrome)	KCNE1L
Paraneoplastic neurologic disorders	PNMA1
X-linked mental retardation with growth hormone deficiency	SOX3
Eye malformations	
Primary congenital glaucoma, autosomal dominant iridogoniodysgenesis anomaly, and Axenfeld–Rieger anomaly	FOXC1
Optic nerve hypoplasia with syndromic microphthalmia, a severe form of structural eye malformation	SOX2
Developmental disorders	
Swyer syndrome, XY females with gonadal dysgenesis	SRY
Sudden infant death with dysgenesis of the testes (SIDDT)	TSPYL
Sertoli cell-only syndrome and male infertility	USP26
Prader–Willi syndrome (contiguous gene syndrome) hypogonadism, hypotonia, obesity	NDN (necdin)
Williams syndrome, a multisystem developmental disorder	DNAJC30
Proximal symphalangism (SYM1)	NOG
Cancer	
Colorectal carcinoma and renal cell tumors	CLDN8
Familial melanoma or familial chronic lymphocytic leukemia (CLL)	ARLTS1 (tumor suppressor)
Myelodysplastic syndrome and acute myelogenous leukemia	PURA
T-cell acute lymphocytic leukemia 2 gene and T-cell acute lymphoblastic leukemia	TAL2
Other	
Hermansky–Pudlak syndrome type 6	HPS6
Fanconi anemia	FANCF

transcriptase. These activities enable self-transposition, but also the propagation of short interspersed elements (SINEs: Alu and SVA) that cannot self-propagate, and occasionally transposition of normal mRNAs [17]. Retrocopies of cellular mRNAs are not necessarily "dead on arrival"; they may accumulate mutations and degenerate into processed pseudogenes or retain an intact coding sequence and become a functional, intronless retrogene. To be expressed in the new genomic location, a retrocopy acquires regulatory elements that promote their transcription. This acquisition is usually achieved by retroposition in the vicinity of some pre-existing regulatory elements (into the intron of a host gene, near a promoter or proto-promoter not previously associated with genes, by the recruitment of some distant regulatory elements, or *de novo* evolution of a promoter). Alternatively, promoter sequences can be inherited from parental genes with multiple transcriptional start sites [19].

Retroposition favors chromosome X: there are a non-proportionally large number of insertions into or out of that chromosome [19]. The "out of the X" preference in retroposition can be explained by meiotic sex chromosome inactivation (MSCI) during spermatogenesis; X-linked parental genes are transcriptionally silenced but their autosomal retrocopies are fully active. Via this mechanism, retrogene expression may rescue a deficiency in parental gene expression. Autosomal retrocopies have been shown to be specifically expressed in testis, whereas their parental genes are often ubiquitously expressed housekeeping genes [20]. Thus, retroposition mechanisms may provide an explanation for the unusually high number of IGs with testis-specific expression.

Many examples of autosomal intronless retrocopies of the X-linked parental genes expressed in the testis are known, including transcription coactivator TAF1L, phosphoribosylpyrophosphate synthetase PRPS1L1, phosphoglycerate kinase PGK2, and pyruvate dehydrogenase PDHA-2. Many examples are also available for other functional intronless retrocopies that exert the same or similar activity as parental genes, but their expression is tissue-specific, particularly testis or neuro-specific (e.g., glutamate dehydrogenase GLUD2 and cell division cycle protein CDC14C). Some IGs encode proteins with the same activity but cellular localization different from the protein encoded by the parental gene (e.g., mitochondrial ferritin, FTMT, as opposed to cytosolic ferritin encoded by the intron-bearing gene FTH1 [21]).

In addition to promoting their own transcription, retrogenes face one more problem on the route to expression: they cannot use a splicing mechanism for efficient processing and exportation out of the nucleus. The L1 element itself was shown to use a specific region in its 3′UTR, named L1-NBE, to bind nuclear export protein TAP (NXF1) [22]. As described below, this method of hijacking cellular mechanisms to bypass the necessity of splicing was also used in other instances, but studied examples are scarce.

6. Processing of intronless mRNA and the potential impact on reporter studies

The biogenesis of mRNA in higher eukaryotes requires several steps of processing: 5′ capping, 3′ cleavage and polyadenylation, splicing, regulation of transcript stability, nuclear export, cytoplasmic transport, and regulation of translation. These steps are often functionally coupled, and splicing in particular seems to be central to all of them [23,24]. Splicing factors cover most of the transcripts in higher eukaryotes and provide access to the nuclear export machinery. The most important factors in this process are UAP56 and Aly, which bind to nucleoporin-binding protein TAP (NXF1), enabling mRNA transfer through the nuclear pore. Splicing factors, especially those forming an exon–junction complex, are also essential for the subsequent NMD of some transcripts. Transcripts of IGs in higher eukaryotes do not have immediate access to these pathways, and they need to find alternative means for processing. Accordingly, the intronless transcripts studied to date seem to be NMD-insensitive [25,26]. However, the issue of their nuclear export has apparently been solved because they effectively accumulate in the cytoplasm and are stable, whereas size-matched

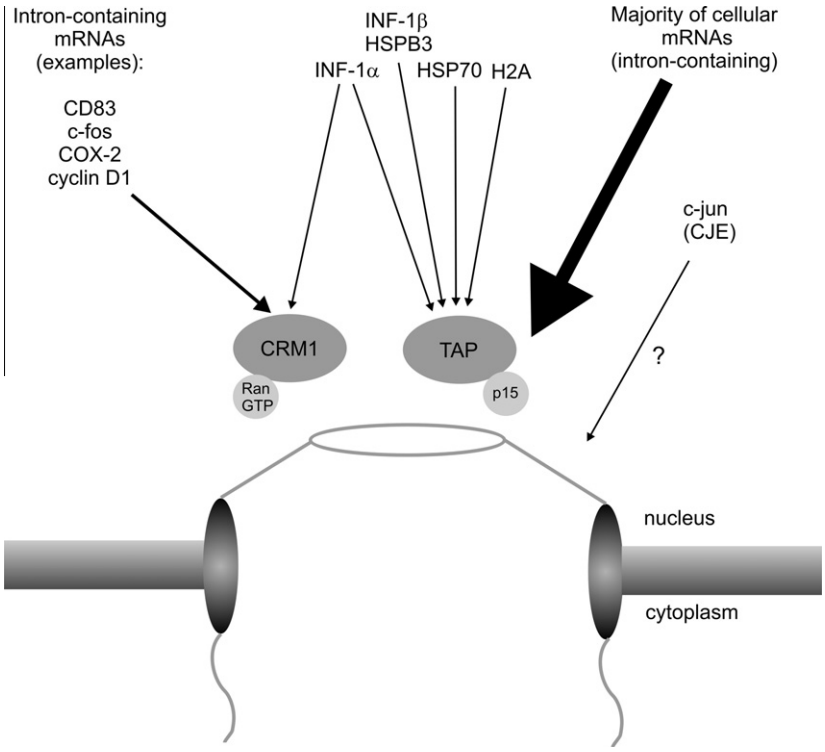

Fig. 1. Nuclear export of mRNA. The majority of transcripts (intron-containing and intronless) exit the nucleus via the TAP pathway. CRM1-mediated export was demonstrated for the indicated transcripts. The export route has not been determined for c-Jun mRNA.

transcripts with a random sequence remain in the nucleus and are highly unstable [27].

The problem of processing and exporting unspliced mRNAs has been efficiently solved several times by various viruses, in which the life cycle demands an export of unspliced or partially spliced messengers. Several cis-acting elements promoting nuclear export have been identified in viral transcripts, including the post-transcriptional regulatory element (PRE) of hepatitis B virus (HBV) [28], the pre-mRNA processing enhancer (PPE) of herpes simplex virus' thymidine kinase (HSV-TK) [29], the constitutive transport element (CTE) of Mason–Pfizer monkey virus (MPMV) [30], the direct repeats (DR) of the Rous sarcoma virus (RSV) [31], and the Rev-responsive element (RRE) of the human immunodeficiency virus (HIV) [32]. These elements were shown not only to enhance nuclear export, but they often have an impact on mRNA stability and 3′ end processing. This impact was significant when the element was derived from naturally intronless viral genes (PRE, PPE) and not observed for intron-containing genes (RRE, CTE) [33].

CTE directly binds TAP [34], whereas PRE binds it indirectly through viral adaptor protein ICP27 [35]. These reports suggest that viral intronless mRNA export is mediated by a modified TAP pathway. However, RRE-containing HIV transcripts also use an alternative route via viral protein Rev, which binds to exportin 1 (XPO1, CRM1). CRM1 is a Ran-GTP-dependent karyopherin responsible for the nuclear export of many proteins, but also some RNA species (rRNA, U snRNA, and a few cellular mRNAs). From the available data, the role of CRM1 in mRNA export seems to be marginal, but examples exist of important cellular mRNAs that use this alternative export route [36–40].

Only a few cellular intronless mRNAs have been studied in respect to their nuclear export; they encode HSPB3 (small heat shock protein), IFN-α1, IFN-β1 (interferons), Hsp70 (heat shock protein 70 kDa), mouse histone H2A, and proto-oncogene c-Jun. As expected, most of these proteins use the TAP exit route [27,41,42]. For the c-Jun transcript, this was not determined. In genes encoding HSPB3, IFN-α1, IFN-β1, H2A, and c-Jun, part of the coding regions were identified as being responsible for nuclear export [27,33,43]. The c-Jun element, called c-Jun gene's enhancer (CJE), was shown to enhance not only nuclear export, but also the stability and 3' end processing of the intronless beta-globin reporter [33].

H2A mRNA was shown to bind members of the SR family of splicing factors, which mediate interactions with TAP [44]. However, histone transcripts should be considered separately because they undergo completely different processing due to a lack of polyadenylation. The 3′ end processing consists only of cleavage, leaving the stem-loop element instead of a polyA-tail. This stem-loop element recruits stem-loop binding protein (SLBP) and other histone-specific processing factors. Therefore, the histone mRNA approach to nuclear export is probably histone-specific, though it conforms to a general rule of recruiting TAP-binding adaptors.

Although hijacking the TAP pathway seems to be a common technique adopted by intronless transcripts to exit the nucleus, a slight discrepancy is seen in the existing studies regarding the role of CRM1 in this process. As depicted in Fig. 1, all but one of the studied mRNAs, including INF-α1 mRNA, were shown to use the TAP pathway, but an earlier study by Kimura et al. [36] demonstrated CRM1-dependent nuclear export of IFN-α1 mRNA. Both exit routes seem to be used by different transcripts and even the same transcript may exercise both pathways, depending on the cell status and factors such as stress or infection. Notably, a number of CRM1-dependent mRNAs, have been isolated from the Jurkat T cells after activation [40].

Finally, one may contemplate the impact that the absence of splicing has on the expression of intronless cDNAs, widely used in molecular studies. Being intronless, cDNAs face even bigger problems with mRNA processing than naturally intronless mRNAs because, as recapitulated above, the latter possess specific adaptor-binding elements, enabling them to use one of the cellular

pathways. Reporter studies examining intron effects on mammalian gene expression, although displaying great variability in the effects [45], have been quite controversial regarding the effect of splicing on nucleocytoplasmic distribution [46–49]. A recent study [50] showed that splicing promotes nuclear export, enhancing the expression of spliced mRNAs 6- to 10-fold relative to their cDNA counterparts.

Which export pathway is used by these cDNA products is not exactly known. A report by Kimura et al. [51] demonstrated that firefly luciferase mRNA nuclear export requires CRM1, but another route also has been suggested [52]. Determining the exit routes for cDNA transcripts may improve experimental design and contribute to the elimination of discrepancies. Knowledge acquired from the studies of natural IGs may contribute to the efficiency of cDNA expression–as proposed by Guang and Mertz [33], the inclusion of adaptor-binding elements like PPE in commercial vectors may considerably improve their performance. On the other hand, splicing affects not only NMD or nuclear export, but also translational yield: the highly intron-dependent β-globin gene was engineered to efficiently accumulate in the cytoplasm (via the insertion of a retroviral export element), but it was still inefficiently translated [48].

7. Summary

Human IGs represent a fascinating group of genes which expression is important for functions as vital as the regulation of growth and proliferation, development, tumorigenesis, and immune responses. The regulation of IGs' expression, especially at the level of mRNA processing, is still much of an enigma. Further studies on the regulation of IGs' expression would contribute to a better understanding of the role of splicing in post-transcriptional events and provide useful information in regard to IGs as therapeutic targets.

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