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The Integrator complex at the crossroad of coding and noncoding RNA

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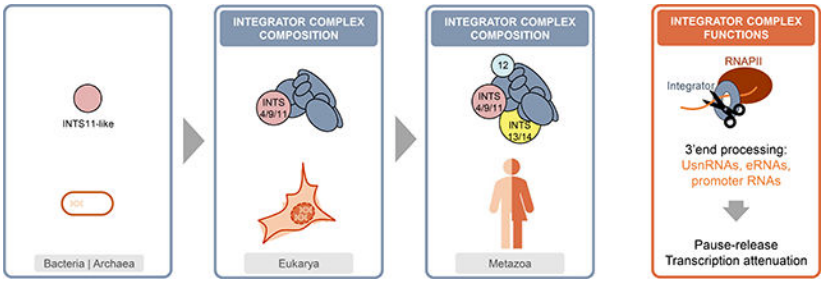
Abstract

Genomic transcription is fundamental to all organisms. In metazoans, the Integrator complex is required for endonucleolytic processing of non-coding RNAs, regulation of RNA polymerase II pause-release, and premature transcription attenuation at coding genes. Recent insights into the structural composition and evolution of Integrator subunits have informed our understanding of its biochemical functionality. Moreover, studies in multiple model organisms point to an essential function of Integrator in signaling response and cellular development, highlighting a key role in neuronal differentiation. Indeed, alterations in Integrator complex subunits have been identified in patients with neurodevelopmental diseases and cancer. Taken together, we propose that Integrator is a central regulator of transcriptional processes and that its evolution reflects genomic complexity in regulatory elements and chromatin architecture.

Graphical Abstract



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Author contributions

Nina Kirstein: Writing – original draft, visualization; Helena Gomes Dos Santos: Formal analysis, visualization, writing – review and editing; Ezra Blumenthal: Writing – review and editing; Ramin Shiekhattar: Conceptualization, writing – review & editing, supervision, funding acquisition

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Introduction

The discovery of the Integrator complex by Baillat *et al.* in 2005 was a major advance in our understanding of the interplay between RNA Polymerase II (RNAPII)-mediated transcription and the maturation of spliceosome-relevant U-rich small nuclear RNAs (UsnRNAs) [1]. This ~1.5 MDa multimeric protein complex is specific to higher eukaryotes, contains at least 15 subunits including SEM1 (also known as DSS1), and tightly interacts with the RNAPII C-terminal domain (CTD) [1–4]. The pivotal subunit to Integrator function is Integrator subunit (INTS) 11, which contains a metallo-β-lactamase domain with endonucleolytic activity. Together with INTS9 and INTS4, INTS11 forms the core catalytic complex of Integrator ('cleavage module') [5]. INTS11/INTS9 are homologous to each other and to members of the cleavage and polyadenylation specificity factor (CPSF) complex CPSF3/CPSF2 (also called CPSF73/CPSF100) [1,6] that are required for maturation and polyadenylation of pre-mRNAs [7].

Since the initial identification of Integrator's role in UsnRNA processing [1,8,9], Integrator's function in the regulation of the non-coding transcriptome has been further expanded to the 3'-end maturation of enhancer RNAs (eRNAs) [10], long non-coding RNAs (lncRNAs) [11], including *NEAT1* [12], and the human telomerase-associated RNA *TERC* [13]. Recently, Integrator was reported in the regulation of coding transcription, from transcriptional activation [14,15], the control of RNAPII pause-release [14,16–19], to transcription termination [20]. These various roles of the Integrator complex impact on cellular homeostasis on multiple levels with alterations implicated in neurodevelopmental defects [21–23] and cancer [24,25].

We discuss in this review the evolution of INTS in multicellular organisms, with a particular focus on the homologs INTS11/INTS9 and CPSF3/CPSF2, as well as recent structural studies that have provided insights into subunit interactions and functions. We further outline Integrator's roles in transcriptional regulation of the non-coding and coding transcriptome and discuss recent key findings that not only advance our knowledge of how Integrator modulates transcriptional homeostasis, but also how INTS alterations mediate pathophysiology.

Integrator evolution parallels that of genomic complexity

The Integrator complex is confined to multicellular animals (Metazoa) [1,26]. Using gene age estimates based on publicly available ortholog databases revealed the emergence of most INTS at the origin of eukaryotes (nucleated cells, Figure 1a, [27]). Later, full-length INTS13, INTS5, INTS10, and INTS14 evolved with multicellularity at the level of Eumetazoa (animals, except sponges). INTS12 was the latest arrival to the complex in Bilateria (bilateral body symmetry and three germ layers, Figure 1a). Interestingly, this subunit contains a plant homeodomain (PHD) finger, a domain generally referred to as epigenetic reader because of its recognition of histone H3 modifications [28], thus linking Integrator complex to chromatin.

When screening more than 1000 eukaryote reference proteomes [29], we identified over 300 species with homologous proteins to human Integrator subunits within our cut-offs of 30% sequence identity and 50% protein coverage to the full-length human INTS (Figure 1b). The majority (70%) of the retrieved species belonged to the animal kingdom, followed by plants (18%), other eukaryotes (10%), and fungi (2%). Integrator is largely absent in fungi, including *Saccharomyces cerevisiae*, suggesting that other RNA processing factors function in UsnRNA processing [30]. In plants (*Arabidopsis thaliana*), the DSP1–4/CPSF73-l complex identified as being responsible for UsnRNA maturation contains subunits homologous to INTS7, INTS3, INTS4, and INTS9, respectively, while the catalytic subunit seems to more closely resemble CPSF3 than INTS11 [31].

Homologues of the catalytic subunit INTS11, containing the three-domain architecture metallo-β-lactamase, β-CASP, and Zn-dependent metallo-hydrolase RNA specificity domain (RMMBL) [32] (Figure 1c), are commonly detected in bacteria and archaea (Figure 1a). The protein phylogeny of the metallo-β-lactamase superfamily revealed at least two duplication events from a common ancestor for the paralog proteins INTS11/CPSF3 and INTS9/CPSF2, suggesting a loss of cleavage activity in the INTS9/CPSF2 branch (Figure 1c, [33]). The two catalytic proteins CPSF3/INTS11 are found most conserved within their orthologous groups, consistent with their functional relevance, while CPSF2 and INTS9 are more divergent (Figure 1c). The heterodimeric interaction between INTS9 and INTS11 is mediated through their conserved C-termini, which represent distinguishing differences between the Integrator complex and the CPSF complex [6].

Novel structural insights indicate Integrator's modularity

Since INTS are essential for life, most studies utilize RNA interference (RNAi) targeting the panel of INTS to deduce functions of individual Integrator components. However, technical difficulties resulting from knock-down efficiencies in different species and disrupting the integrity of the complex following knock-down provide barriers to assigning function for individual subunits. Therefore, elucidating Integrator's structure would provide further insights into its mechanism of action and its modular nature. The C-terminal interaction INTS9/INTS11 was the first to be structurally resolved [34]. More recently, the INTS13/INTS14 heterodimer structure was determined and found to form a subcomplex together with INTS10 [35]. Interestingly, all three subunits appear at the origin of Eumetazoa (animals, except sponges). This subcomplex was described to interact with the cleavage module (INTS4/INTS9/INTS11) through INTS13, and has affinity to single-stranded RNA hairpin structures. These observations provoke the model of INTS10/INTS13/INTS14-RNA interactions recruiting the Integrator cleavage module and forming a platform for remaining Integrator subunits for efficient RNA processing ([35] and Figure 1d).

By contrast, specific INTS interacting with RNAPII CTD, as well as requirements for their interaction remain largely elusive. The CTD consists of a number of tandem heptapeptide repeats (Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7) that varies according to the organism. Its phosphorylation code changes dynamically throughout the transcription cycle, exhibiting distinct patterns for initiation, elongation and termination (reviewed in [36]). For UsnRNA genes, Integrator-CTD interaction seems to strictly depend on the CTD phosphorylation

status and requires both Ser2-phosphorylation (Ser2-P, productive elongation) and Ser7-P (promoter located) [2]. Interestingly, interactome studies by mass spectrometry find equally strong Integrator interaction with both hypo-phosphorylated and hyper-phosphorylated CTDs [3], arguing for a general presence of Integrator at RNAPII-engaged genes independent of its phosphorylation status. Recently, Integrator was also found to interact with Tyr1-P CTD, which is required for RNAPII pausing and transcription termination [4].

Both the non-coding and coding transcriptomes rely on Integrator function

Early work identified a degenerate A/T-rich consensus sequence ($GTTTN_{0–3}AAARNNAGA$) downstream of the UsnRNA 3'ends termed the 3'box, which is required for efficient UsnRNA processing [37]. Chromatin immunoprecipitation (ChIP) experiments co-localized RNAPII and Integrator at UsnRNA promoter, gene body, and 3'end of U2 snRNA, indicating that Integrator associates with RNAPII constitutively throughout transcription [9]. Furthermore, knock-down of various Integrator subunits leads to UsnRNA extensions beyond the 3'box, suggesting that Integrator recognizes the 3'box and UsnRNA hairpin structure signals for cleavage of the transcripts [1,8]. Moreover, primary miRNA hairpin structures originating from the γ-herpesvirus *Herpesvirus saimiri* require Integrator for their processing into miRNA precursors using a similar 3'box-like recognition sequence [38].

The scope of Integrator's action on non-coding RNAs was further expanded by its identification as a crucial factor for eRNA maturation and enhancer-promoter communication. Lai *et al.* focused on a set of epidermal growth factor (EGF) activated enhancers of immediate early genes (IEGs), and found a requirement for Integrator in the 3'end cleavage and termination of primary eRNA. Additionally, Integrator was deemed essential for EGF-induced chromatin looping, as identified by chromosome conformation capture [10]. Finally, it was shown recently that Integrator displays a global role in eRNA processing at most active enhancers [10,19].

Transcription is regulated at multiple levels to precisely execute gene expression programs critical for cellular homeostasis, development, and response to environmental cues. Besides Integrator's function in enhancer-promoter looping and subsequent gene activation, it also acts on recruitment of RNAPII to promoters [14,19], premature transcriptional attenuation [17,18,20], and RNAPII pause-release followed by transcriptional elongation [14,16,19]. Advances in nascent RNA sequencing further accelerated our understanding of Integrator's role in transcription. Two recent studies in Drosophila and human sought to decipher Integrator's regulatory mechanisms at gene promoters using precision run-on sequencing (PRO-seq), a method that maps actively engaged RNAPII at single nucleotide resolution [17,19]. While in Drosophila, INTS9 depletion leads to activation of a small subset of genes displaying enhancer-like chromatin modifications [17,18], human Integrator plays a more profound and wide-ranging role in the regulation of gene expression [19]. Here, INTS4, INTS9, or INTS11 depletions lead to equal proportions of gene activation and repression, which could be attributed to distinct mechanisms by which Integrator regulates transcriptional initiation and subsequent regulation of transcriptional elongation beyond the +1 nucleosome [19]. While standard PRO-seq analyses approaches like the traveling ratio or

pausing index are limited in resolving RNAPII transcriptional dynamics at promoter and gene body, the establishment of the traveling matrix significantly improved our understanding of Integrator in transcriptional regulation (Figure 2a). Separating positional changes in RNAPII at promoters and gene bodies incurred by the loss of INTS11 led to the identification of four gene classes. While Integrator depletion leads to transcriptional de-repression in class I and II genes (33%), 67% of genes (class III and IV) require Integrator for their transcriptional activity (Figure 2a). Additionally, while class I and III genes rely on Integrator for RNAPII recruitment to the promoter, class II and IV genes require INTS11 cleavage activity for proper regulation [19]. Consequently, cleavage of stalled transcripts by INTS11 at these genes leads to premature termination of transcription in the proximity of +1 nucleosome, allowing for productive transcriptional elongation by subsequent rounds of initiation (Figure 2b, [19]). Conversely in Drosophila, Integrator was shown to prematurely terminate transcription at a small subset of genes (~15%) displaying unusual histone modifications of high levels of mono-methyl H3K4 and low levels of tri-methyl H3K4 in the process termed attenuation leading to decreased transcription (Figure 2c, [17]).

Interestingly, INTS8 was recently identified to interact with the protein phosphatase 2A (PP2A), which is required for dephosphorylation of RNAPII CTD and pausing factor Spt5 [39], thus inhibiting productive elongation. This study emphasizes the importance of transcriptional regulation mediated by the Integrator complex, albeit independent of its endonucleolytic activity.

What links Integrator malfunctions to neurodevelopmental defects and cancer?

As a result of Integrator's essential functions, the full disruption of the complex is lethal during embryogenesis [40,41]. Strikingly, individuals harboring biallelic mutations in INTS1 and INTS8 exhibit serious neurodevelopmental defects [21,22]. Integrator was also identified to be crucial for ciliogenesis, the formation of primary cilia responsible for signal transmission [42]. Dysfunctional ciliary proteins are the cause of ciliopathies, which manifest as defects in organogenesis as well as cognitive deficits related to neurodevelopmental disorders. Indeed, INTS6 and INTS10 were identified in a CRISPR based screen as novel ciliopathy genes, linking Integrator and Hedgehog signaling during mammalian ciliogenesis [43].

Multiple recent studies used *in vivo* or *in vitro* models to pinpoint the mechanism of Integrator action during embryonic development. Oegema *et al.* suggest that INTS8 mutations lead to Integrator complex disruption, resulting in UsnRNA misprocessing, and altered splicing patterns. Consequently, resulting gene expression changes lead to impaired neurodevelopment potential [21]. INTS expression peaks during early development, such that INTS RNAi reduces the organism's differentiation capacity in flatworm *Schmidtea mediterranea* [44] or brine shrimp *Artemia sinica* [45]. In mouse, the absence of Integrator leads to neuronal migration defects, an underlying cause of neurological disorders [46]. More interestingly, Van den Berg *et al.* demonstrate Integrator's presence at active and poised promoters and enhancers of the relevant gene network, placing Integrator at the

junction of signaling and gene expression [46]. Indeed, Integrator-dependent gene regulation has been widely reported as a response to environmental stress [18,45,47] and signaling induction [14,15]. This connection insinuates context-specific interactions of Integrator with transcription factors (TFs), as reported for the fibroblast growth factor downstream TF Esrrb in trophoblast stem cells during self-renewal [48].

Consistent with a fundamental role in modulating gene expression pathways during signal transduction, Integrator mutations were also detected in multiple cancers [25]. Interestingly, lower expression levels of multiple INTS correlate with poorer overall patient survival in various cancer cohort analyses [12]. Additionally, INTS3 was found to be mis-spliced in acute myeloid leukemia [49], resulting in the loss of further INTS and the cells' differentiation potential, in accordance with observations during development. Finally, a negative selection genetic screen (purifying selection) for mutations in essential genes for cancer identified INTS10 as highest ranked gene, placing Integrator as potential target for cancer treatment [50].

Conclusion and outlook

While the Integrator complex evolved with the appearance of the nucleated organisms, its composition and potential functions in transcription increased with genome complexity, consistent with Integrator's requirement in development. Additionally, some INTS form smaller subcomplexes [1,35,51], and INTS3 and INTS6 have been also shown to participate in DNA damage response [51]. Similarly, INTS13 represents an independent submodule targeted to poised enhancers [52]. Understanding the structural modularity and assembly of the complex, as well as its interaction with RNAPII (Box 1) will further enhance our understanding of Integrator functions and allow identification of potential interfaces for therapeutic interference.

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Box 1:

Future perspectives

To date, many aspects of Integrator's assembly and functions remain elusive.

- Beyond the catalytic module, what are other sub-modules of Integrator complex?
- Is Integrator interaction with RNAPII CTD conserved throughout evolution? And which INTS mediate this interaction?
- What is the mode of Integrator recruitment to various promoters?
- What is the mechanism of Integrator effects on chromatin architecture?
- How does Integrator contribute to DNA repair?
- What are the determinants of RNA cleavage by INTS11 and how other Integrator subunits contribute to RNA cleavage activity?
- Why Integrator mutations in humans are manifested by neurodevelopmental disorders?

Highlights

- The Integrator complex originates in Eukarya and gains additional subunits in parallel to increasing organism/genome complexity
- Integrator is required for transcription homeostasis: from 3’end processing of functional small RNAs, to regulation of RNA polymerase II pause-release and premature transcription termination
- Alterations in human Integrator subunits contribute to neurodevelopmental defects and cancer

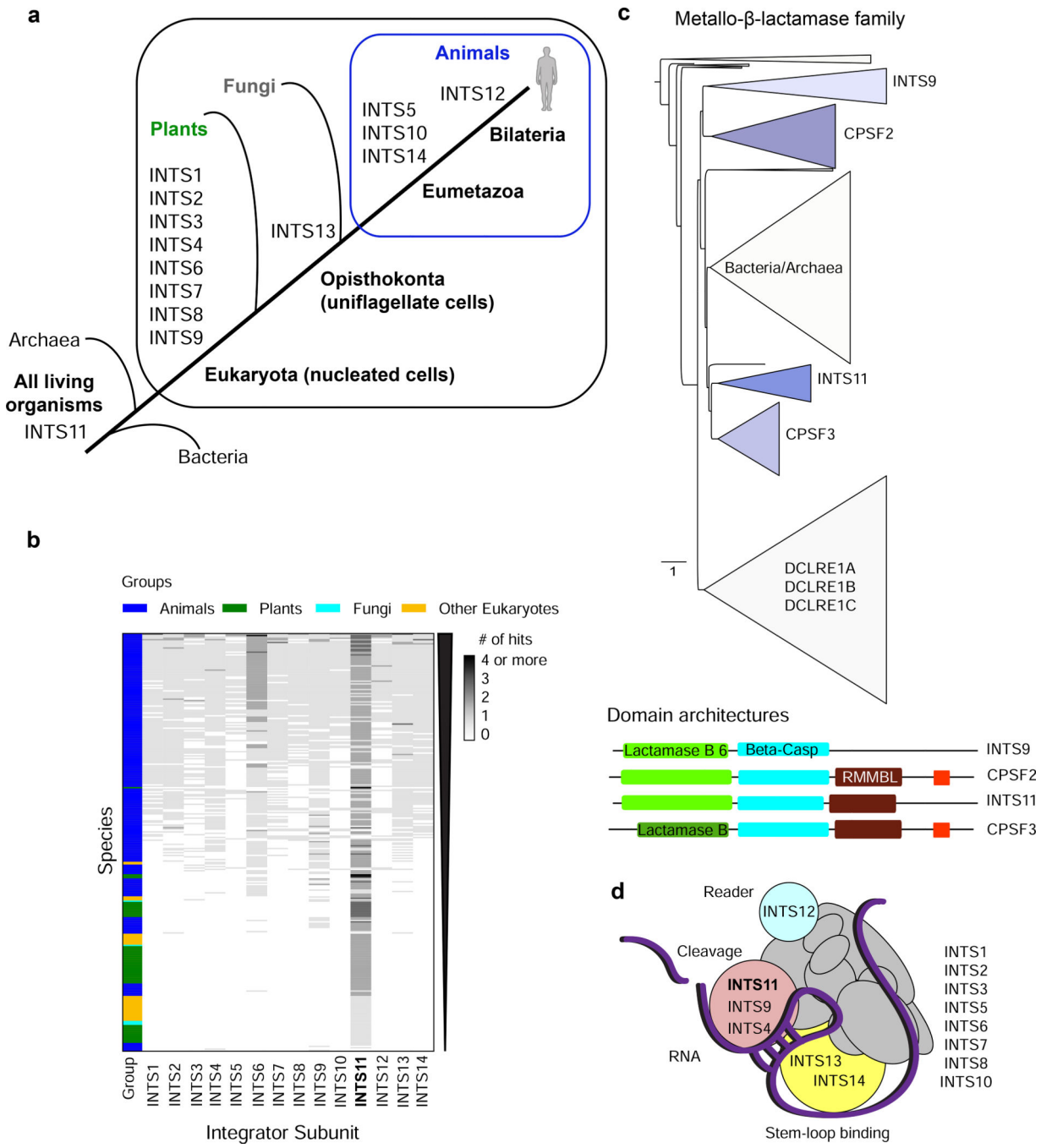

Figure 1. Evolution of the Integrator complex.

a) Overview of the INTS gene ages throughout evolution. Eukarya (nucleated cells), Opisthokonta (uniflagellate cells), Eumetazoa (animals, except sponges), Bilateria (animals with bilateral body symmetry and three germ layers). b) Heatmap of the presence/absence of Integrator-homologous proteins in >300 species (compared to full-length human sequences with the following cut-offs: 30% pair-wise sequence identity, and 50% coverage of the human proteins, in addition to blastp default parameters [29]). Grayscale accounts for the number of hits in one species that aligned to a human INTS. c) Phylogenetic tree of the

metallo-β-lactamase superfamily from eggNOG 4.5.1 (COG1236) [33]. Triangle length indicates divergence and triangle height the number of species. Below: domain architecture of human INTS9, CPSF2, INTS11, and CPSF3, as retrieved from Pfam 33.1 [32]. In red CPSF73–100_C domain. d) Model of INTS and their modular interactions. Cleavage module depicted in pink, stem-loop binding module in yellow, and reader module in cyan. Bold: catalytic subunit INTS11.

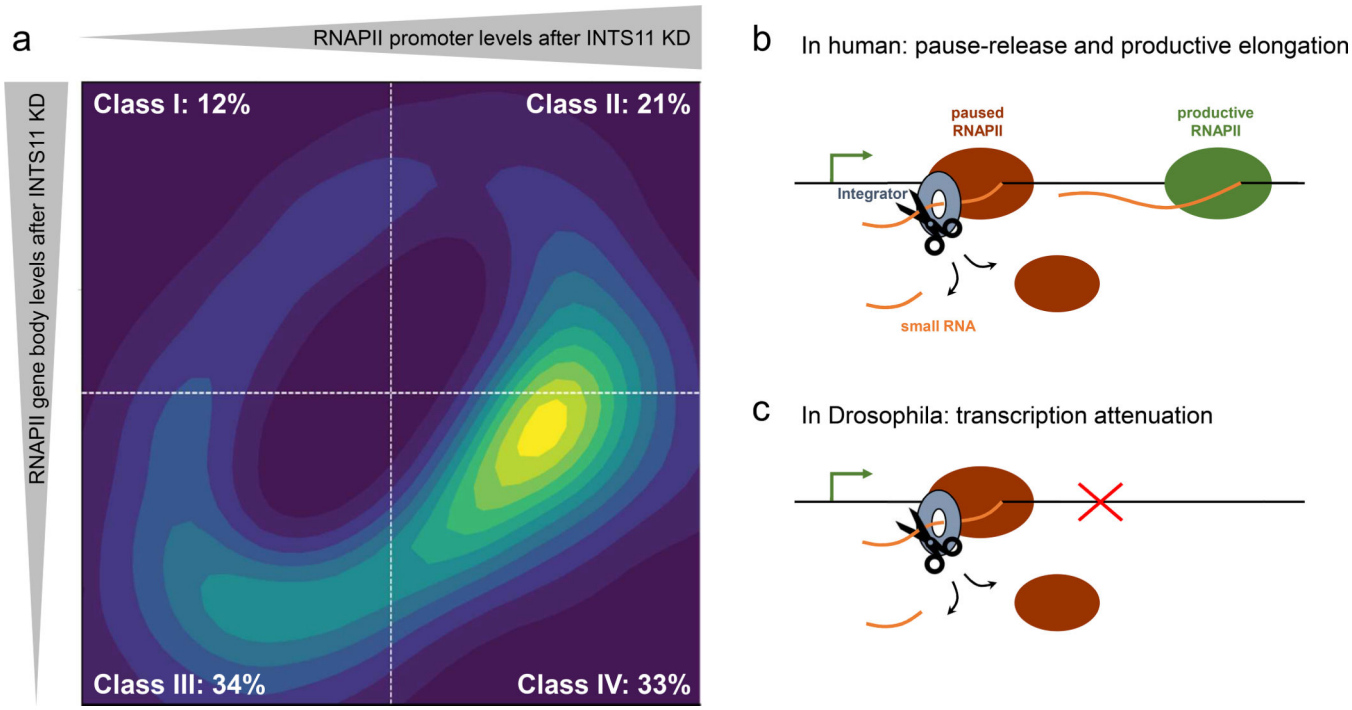

Figure 2. Integrator's main functions on coding genes in human and Drosophila.

a) The travelling matrix separates positional RNAPII changes at promoters and gene bodies into four classes. Graphical depiction of ~3100 significant Integrator-responsive genes [19]. In human, INTS11 depletion predominantly leads to downregulation of actively engaged RNAPII (class III and IV: 67%). Class IV genes are additionally characterized by increased RNAPII pause (33%). b and c) Model representation of Integrator's functions in human and Drosophila. b) The Integrator complex cleaves promoter-associated small transcripts to allow paused RNAPII eviction and transcriptional elongation by productive RNAPII (class IV). c) Integrator is required for the premature termination (attenuation) of RNAPII transcription.