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Mobile self-splicing introns and inteins as environmental sensors

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Abstract

Self-splicing introns and inteins are often mobile at the level of the genome. Although these RNA and protein elements, respectively, are generally considered to be selfish parasites, group I and group II introns and inteins can be triggered by environmental cues to splice and/or to mobilize. These cues include stressors such as oxidizing agents, reactive oxygen and nitrogen species, starvation, temperature, osmolarity and DNA damage. Their sensitivity to these stimuli leads to a carefully choreographed dance between the mobile element and its host that is in tune with the cellular environment. This responsiveness to a changing milieu provides strong evidence that these diverse, self-splicing mobile elements have adapted to react to prevailing conditions, to the potential advantage of both the element and its host.

Introduction

In the 1980's, with the discovery of mobile self-splicing introns in T4 bacteriophage, we conjectured that these selfish elements might play a regulatory role in gene expression [1,2]. We posited that these group I introns, which interrupt genes in a common pathway of nucleotide metabolizing enzymes, have the ability to act as sensors. Could introns be responsive to cellular and environmental cues, in ways that may be advantageous to their hosts? Little did we know then that the search for direct evidence would follow such a long and winding road.

Self-splicing mobile introns and inteins are usually bipartite, composed of both the catalytic splicing apparatus and a protein required for mobility. An RNA ribozyme is the splicing catalyst of group I and group II introns, whereas a protein enzyme comprises the self-splicing intein (Fig. 1A). Group I introns and inteins use their associated homing endonucleases (HEs) to cleave DNA at precise targets and mobilize by homing through a double-strand-break mediated pathway (Figs. 1A & 1B) [3–5]. In contrast, group II introns move by an RNA-based retrohoming pathway. The excised intron reverse splices into DNA, in site-specific fashion, guided by base-pairings between the intron and the DNA. The

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integrated intron is then copied by the intron-associated reverse transcriptase (RT) (Figs. 1A & 1C). A primer for the RT is provided by a nick into dsDNA introduced by HE activity associated with the RT. Alternatively, if integration is into ssDNA, such as at a replication fork, fragments of the lagging DNA strand act as primers (Fig. 1C), resulting in target primed reverse transcription (TPRT) [3,6–10]. Group II introns have also been shown to mobilize less specifically and at low frequency to ectopic sites, usually by the ssDNA pathway, a process known as retrotransposition. There is mounting evidence that all of these mobile self-splicing elements excise in a condition-dependent manner in response to a battery of signals, whereas the introns in particular also mobilize in reaction to various cellular stressors. The emerging picture is one of domestication, and that many of these invasive parasitic elements evolved opportunistically in concert with the host, in both of their best interests.

Environmental triggers regulate group I intron self-splicing and mobility

Group I introns can have their splicing rate stimulated or inhibited, or their splicing pathways regulated by different environmental cues (Fig. 1A). There are instances of chloroplast group I intron splicing being triggered by light, which is precisely the condition that requires the host gene product [11]. The mechanism of light-induced pre-RNA processing is unclear, but stimulation is affected by photosynthetic electron transport, which may impact the redox status of a protein that facilitates splicing. In contrast, there are several examples of bacterial and organellar group I introns that have splicing inhibited by a range of conditions. Inhibitory factors include small molecules, such as flavin mononucleotide (FMN) [12]. FMN acts as a competitive inhibitor of splicing by interfering with the affinity of the intron for GTP, which is the initiating nucleophile for group I intron catalysis.

For T4 introns, growth state is important and stationary phase inhibits splicing [13]. This observation may be related to protein synthesis, where the act of translation of the upstream exon and the intron open-reading frame (ORF) facilitates splicing [5,13]. This is understandable, given the coupling of transcription and translation. Since much less translation occurs during stationary phase, these phenomena are probably related. Natural phage populations are likely to exist in a stationary state in nutrient-poor environments, where there are few translating ribosomes and phage bursts are delayed [5,13]. Limiting dNTP production by stalling splicing would allow energy to be channeled to cell survival until more favorable conditions are encountered, when splicing and phage development would ensue. This hypothesis harkens back to our early speculation of the regulatory potential of group I introns [1,2].

Growth conditions, such as temperature, can influence the splicing pathway in eukaryotic microbes, where heat-shock increases the circular form of the intron by orders of magnitude, by a yet unknown mechanism [14]. This dramatic increase in circular introns may have implications for mobility, but excised group I introns have never been shown to mobilize by reverse splicing, unlike their group II counterparts. Regardless, these observations collectively suggest that group I ribozymes may function as molecular sensors.

A compelling case of conditional RNA splicing is provided by a group I intron upstream of a possible virulence gene in *Clostridium difficile*, where accurate splicing is regulated by a neighboring riboswitch (Fig. 2). The riboswitch senses the second messenger c-di-GMP [15,16], providing an intriguing example of a riboswitch-ribozyme collaboration: the riboswitch controls 5'-splice-site choice of the group I intron, such that a ribosome binding site for translation of the downstream ORF forms only in the presence of high c-di-GMP levels (Fig. 2). This allosteric ribozyme lacks a HE gene, characteristic of its selfish mobile counterparts in *C. difficile*, suggesting that the intron has been maintained because of benefit to the host.

The single reported example of stress-responsive effects on group I intron mobility is increased promiscuity triggered by exposure of the phage T4 *td* intron to oxidative stress [17]. This effect is transduced via the intron HE I-TevI. A stress-responsive four-cysteine zinc finger in I-TevI provides a fidelity switch resulting in I-TevI specificity changes (Fig. 1D). Treatment with H₂O₂ releases Zn²⁺ from the zinc finger causing indiscriminate DNA cleavage, and suggesting a mechanism for intron dispersal to degenerate sites. Possible advantages of such dissemination under conditions of stress will be discussed below when considering stress-promoted group II intron dispersal.

Self-splicing mobile group II introns also respond to environmental cues

A few examples exist of group II intron splicing being modulated by growth conditions. For the well-studied *Lactococcus lactis* LtrB intron, Ll.LtrB, acidity reduces mRNA levels and inhibits splicing activity [18]. Additionally, the Ll.LtrB intron introduced into *Staphylococcus aureus* is inherently thermosensitive, splicing at 32°C but not 43°C [19]. The temperature sensitivity is likely largely attributable to that of the intron-encoded protein (IEP). These observations indicate that group II introns also sense the environment and modulate splicing.

Group II intron mobility can be highly condition-dependent. Retrohoming appears to be variably sensitive to intracellular Mg²⁺ concentrations. Whereas mobility of the Ll.LtrB group IIA intron is stimulated by intra- and extracellular Mg²⁺ concentrations [20], the *Rhizobium meliloti*, RmInt1, group IIB intron seems less dependent on Mg²⁺ concentration [21].

Generally speaking, retrotransposition to ectopic sites is much more responsive to stimulation by environmental cues than retrohoming. Even pathway choice is influenced by host environment. The Ll.LtrB intron retrotransposes predominantly by a ssDNA endonuclease-independent pathway in *L. lactis*, but by a dsDNA endonuclease-dependent mechanism in *E. coli* (Fig. 1C) [22]. Integration patterns are also different, being polar in *E. coli* and more random in *L. lactis*. Both pathway choice and integration patterns are also affected by growth rate, likely reflecting replication status and compactness of the nucleoid.

Ll.LtrB intron retrotransposition is greatly enhanced by nutritional stress in response to ppGpp and cAMP, alarmones that respectively signal amino acid and glucose starvation [23,24]. These stimulatory effects are mediated at the level of the DNA target, rather than of

expression of the retroelement. The alarmones cAMP and ppGpp could be acting by influencing the openness of the nucleoid, or at the level of replication forks to stimulate reverse splicing into ssDNA [22,24]. Regardless, dissemination is a reaction to stress for both group I and group II introns, with the possibility of genome remodeling and increasing genetic diversity, which may benefit the host. However, these altruistic arguments need to be balanced against the advantage that accrues for the element to escape from a host whose survival is threatened.

Another striking example of how the state of the recipient DNA can influence retrotransposition of the Ll.LtrB intron is the observation that the conjugative relaxase protein, whose gene hosts the intron, stimulates both the frequency and diversity of retrotransposition [25–27]. The stimulatory effect is ascribable to off-target nicking by the relaxase, where the DNA nicks act as foci for reverse splicing and TPRT (Fig. 1E) [25]. Interestingly, splicing of the intron, which is itself the subject of regulation [18], generates the relaxase, which not only promotes conjugation of the plasmid on which it resides, but also stimulates the intron's dissemination. This dual action provides a fascinating example of the functional interaction between two mobile elements to promote horizontal gene transfer [25–27].

Intein self-splicing is regulated by diverse signals

Like introns, inteins also occur in phage. Inteins and introns can even co-localize in the same gene, as was first described for ribonucleotide reductase of *Bacillus subtilis* phage SPβ [28]. We speculated on this “lightning strikes twice” scenario, coming down heavily in favor of the selective advantage hypothesis [29]. Whereas little is known of environmentally responsive intein mobility, intein splicing has recently been shown to be regulated in spectacular ways by external stimuli. These observations likely account for the enigmatic occurrence of inteins, which cluster in the active centers of enzymes in specific protein networks, like the replisome [30,31]. Although host-imposed barriers to the spread of introns and inteins might account for some of these distribution profiles [23,32], sporadic localization also fuels the hypothesis that retention of some inteins might be beneficial under specific environmental conditions. This argument is bolstered by the finding that nonorthologous, functionally equivalent proteins in bacteria and archaea carry inteins, because intein acquisition by these similar functions must have been independent events. ATPase domains are particular hot-spots for inteins, with 70% of all inteins residing in ATPase-containing proteins, and at many different integration sites (Fig. 3A) [33]. Consistent with this distribution bias is the argument that some inteins act as pause buttons that reversibly inhibit specific proteins until appropriate stimuli are sensed.

Indeed, reactive oxygen and nitrogen species [34], redox [35,36], temperature [37], pH [38], salt [39,40] and DNA damage [41] can modulate splicing in ways that suggest inteins can be biosensors (Fig. 3B). Thereby, inteins can respond to stress and allow instantaneous splicing, and thus protein activation, only under ideal conditions for protein function. Three examples presented below have been selected because they provide a clear mechanistic understanding of how inteins serve as stress sensors. They act by disulfide bond formation as a redox

detector (Fig. 4A), extein-intein interactions as a thermal sensor (Fig. 4B), and substrate-induced splicing as a responder to DNA damage (Fig. 4C).

Many inteins splice by cysteine-based chemistry, making some inteins exceptionally prone to redox-sensitive disulfide bond formation. This has been demonstrated to occur between catalytic cysteines of a PolII polymerase intein [36], as well as between the first catalytic cysteine and a cysteine in the upstream extein of a molybdate biosynthesis MoaA intein [35]. These two inteins provide satisfying regulatory examples, both from the deep-sea archaeon *Pyrococcus abyssi* (Fig. 4A). First, the replication of this hyperthermophile needs to be shut down at suboptimal temperatures, conditions that actually favor disulfide bond formation and lock the intein in an inactive state. Second, MoaA is an [Fe-S]-cluster enzyme with oxidoreductase activity that is oxygen labile. By disulfide bond formation, production of mature MoaA can be prevented under hyperoxic conditions, protecting against oxidative damage to the enzyme [35]. The accumulated precursor can then be spliced under reducing conditions into the mature [Fe-S] enzyme when oxidoreductase function is required and the threat of oxidative damage has passed. The intein in MoaA contains an N-terminal catalytic cysteine that forms this disulfide bond to a cysteine in the N-extein three residues from the intein (at C-3). An intein-extein precursor with a similar C-3 was crystallized and the disulfide bond visualized (Fig. 4A). This intein-based regulation is an adaptation utilized by several inteins to the redox environment [35]. Such regulation is the protein equivalent of a riboswitch.

A quite different example of post-translational control was found in the RadA recombinase intein located in the ATPase domain of the hyperthermophilic archaeon *Pyrococcus horikoshii* [37]. Here, the RadA exteins lock the intein in an inactive state, with interactions between distant residues that are close in three-dimensional space (Fig. 4B). High temperature or solution conditions enable splicing through unlocking the intein-extein association. These interactions thereby serve as a thermal sensor, only releasing the intein for full activity at optimal growth conditions for the native organism. This partnership between the intein and its exteins, just as for the MoaA intein above, implies coevolution of the parasitic intein and its host protein, providing another novel means of post-translational regulation.

The most specific example of splicing stimulation was demonstrated recently for the above-mentioned thermosensitive *P. horikoshii* RadA recombinase intein, which splices much faster and more accurately when presented with ssDNA or intracellular DNA damage (Fig. 4C). The response is highly ssDNA-specific, with neither dsDNA nor RNA promoting RadA splicing [41]. The sensitivity of RadA intein splicing to ssDNA is particularly significant in regard to biological function, as ssDNA is the substrate for RadA and signals that the cell needs recombinase activity. Again, the RadA intein, which is subject to regulation by temperature and DNA damage, lacks a HE, suggesting that the intein is maintained because of benefit to the host, and has undergone the transition from selfishness to altruism. Collectively, these studies provide overwhelming support that some inteins have become domesticated to act as sensors that modulate protein function for an immediate activity switch.

Conclusions

The pathways whereby introns and inteins mobilize are highly relevant to their dissemination in nature, but their distribution in extant genomes is largely a reflection of why and how they persist. Although a clear demonstration that intron- or intein-containing organisms have a selective advantage over their intron- or intein-lacking counterparts has been elusive, their differential reactivity under stressful conditions confirms their ability to act as environmental sensors. We suspect that intron- and intein-containing native hosts will eventually be shown to have advantage over their element-free equivalent under exactly these conditions of stress. This reactivity relevant to the growth conditions of the organism challenges the conjecture of the purely selfish nature of these elements. Together the examples presented provide compelling evidence that self-splicing introns and inteins are mobile elements that, rather than being neutrally evolving, have adapted to become responsive to environmental conditions relevant to their macromolecular and cellular hosts, to their mutual advantage.

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Highlights

- Mobile self-splicing introns and inteins have been surmised to play a regulatory role
- There is mounting evidence that these elements respond to environmental cues
- Stressors can affect splicing and induce mobility of self-splicing introns
- Inteins can be stimulated to splice by a spectacular array of stressors
- The emerging picture is one of adaptation of these parasitic elements to their hosts

A. Mobile self-splicing elements

Element	Self-splicing element	Pathway	Mediator	Splicing regulators	Mobility regulators
Group I intron	RNA	Homing (DNA)	HE ^a	Light, Redox FMN, Growth phase, Translation c-di-GMP	Oxidative stress
Group II intron	RNA	Retrohoming (RNA)	RT ^b -HE	pH Temp	Mg ²⁺ Starvation Conjugation
Intein	Protein	Homing (DNA)	HE	ROS/RNS Redox, Temp pH, Salt DNA damage	None known

^a HE = homing endonuclease
^b RT = homing endonuclease

B. Homing

C. Retrohoming

D. Regulation of Endonuclease

E. Regulation of target

Figure 1. Mobile self-splicing elements. A. Overview of self-splicing, mobility and regulation. B. Group I intron and intein homing pathway. The pathway is initiated by a HE encoded by the element (red Pacman symbol). After cleavage of the recipient the element is inherited by double-strand break repair recombination [42]. C. Group II intron retrohoming. The pathway is initiated by reverse splicing of the excised intron into dsDNA followed by nicking of the opposite strand by the intron-encoded HE [7,8]. Reverse splicing can also occur into ssDNA at replication forks

(left). The ssDNA pathway is most common to retrotransposition where reverse splicing is less site-specific. The RT of the intron-encoded protein (IEP) completes cDNA synthesis by a target-primed reverse transcription (TPRT) mechanism. Cellular repair processes complete the reaction [43]. D. Stress-induced group I intron promiscuity. The I-TevI HE has a 4-cysteine zinc finger, which is destroyed by H_2O_2 leading to spurious cleavage [17]. E. Elevated group II intron retrotransposition. The conjugative relaxase (green Pacman) encoded by the *ltrB* gene that contains the LtrB intron causes elevated retrotransposition by spurious DNA nicking, which nucleates reverse splicing [25].

Figure 2.

Group I intron splicing regulation by riboswitch. The riboswitch preceding the group I intron is activated by c-di-GMP which results in a splicing event that forms a ribosome binding site (RBS, red) for translation of the downstream ORF [15,16]. In the absence of c-di-GMP, splice site selection leads to a truncated product with a vestigial RBS and limited translation of the ORF.

Figure 3.

Overview of intein splicing regulation.

A. Intein clustering. Intein insertion sites (a-e) in the ATPase domain of mycobacteriophage terminase TerL. To the right is a structure model of TerL showing the intein insertion sites a-e at red space-filled residues [33]. B. Conditional protein splicing (CPS). Natural examples of CPS regulated by redox, ROS/RNS, temperature, DNA damage, salt and pH are shown. Intein, red; N-terminal extein, blue; C-terminal extein, green; cysteines, yellow.

Figure 4.

Redox control, thermoregulation and DNA damage control of protein splicing.

A. Redox controlled switches. Disulfide bond formation between Cys1 and Cys-3 in the upstream extein (above) [35], and Cys1 and Cys+1 in the downstream extein (below) [36] are shown. A crystal structure of a Cys1/Cys-3 disulfide is shown in the inset [35]. Colors as in Figure 3B. B. Thermoregulation. Model of extein-intein interactions between RadA and the intein active site are on the left with the amino acid residues involved in the interactions schematized on the right [37]. C. DNA damage regulation is transduced by ssDNA. The RadA precursor (top) interacts with ssDNA to stimulate splicing (middle) that results in ligated exteins (RadA-ATPase) which interacts with and hydrolyzes ATP [41]. The two nucleophiles are Cys1 (pink), and Thr+1 (green). A DNA-binding residue is in dark green.