Functional Diversity and Applications of Mobile Group II Introns

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Abstract *Bacteria*, *Archaea*, and *Eukarya* include numerous thermophiles that are ubiquitous and have been detected in a variety of environments covering a really broad range of temperatures among factors. This suggests a great adaptability to both environmental conditions and nutrient sources which places thermophiles as a major target for environmental and evolutive studies with a great biotechnological potential as source for thermophilic enzymes and the biodegradation of various recalcitrant pollutants. While growth under optimal laboratory conditions is well studied, the potential for thriving under nonoptimal conditions, far from those considered ideal for a microorganism, remains to be studied. This chapter highlights possible development of novel methodology for the analysis of thermophilic microorganisms, which is applicable to other organisms, under natural conditions and for a broad range of extreme environments ranging from cold to hot temperatures, water activity, pH, and salinity as major naturally occurring extreme events. The mobile group II intron (MGI) functional diversity and abundance are assumed to represent a key feature indicator for the use of the full potential of microbial enzymes and a basic physiological process needed to understand microbial capabilities to grow and thrive under extreme conditions.

1 Mobile Group II Introns: A Novel Tool for Biotechnology

Mobile group II introns (MGIs) are "autocatalytic (self-splicing) genetic elements—'ribozymes'—found in bacterial and organellar DNAs of few eukaryotic organisms." These are evolutionary ancestors of spliceosomal introns, retrotransposons, and spliceosomes and in higher organisms, i.e., eukaryotes.

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G. Arora et al. (eds.), *Drug Resistance in Bacteria, Fungi, Malaria, and Cancer*,
DOI 10.1007/978-3-319-48683-3_6

These lines present a novel and promising perspective to better understand the ubiquity, genetic diversity, and abundance of microorganisms under the broadest range of environmental conditions described on Earth. This chapter focuses on understanding the diversity and potential of MGI and their relationship to comprehend the microbial capacity to conquer a wide range of environments, including those representing extreme conditions and the ability of microorganisms to thrive and adapt to suboptimal environmental conditions. A listing of currently known group II introns in microorganisms is presented in later part of this chapter. This clearly indicates the broad distribution of these introns in prokaryotes and induces to believe that a large number of additional MGI can be discovered and described in the next years through extensive investigation based on new-generation sequencing platforms and the huge quantities of high-quality sequence data that is becoming available at the present time. Understanding the potential for the environmental and biotechnological application of microorganisms and specifically of their group II introns is a matter for the development in the next few years (Zimmerly 2014), and it deserves to be highlighted.

2 Current State of Mobile Group II Introns

Some research has been carried out on MGI from extreme environments worldwide, but there is scarce work performed on MGI functional diversity and its consequences for microbial growth, evolution, and their applications. Toro et al. (2007) studied the mobility of bacterial MGI and their splicing mechanisms. They have described reported gene-targeting-based recent development in MGI research. They have also discussed on bacterial MGI, phylogeny, and behavior of MGI in prokaryotes (bacteria). Dai and Zimmerly (2002) suggested that bacterial MGI behaves like retroelements, and their fundamental strategy is different from introns found in eukaryotes. Lambowitz and Zimmerly (2004) described the development of programmable and target-specific MGI into "targetrons." Jones et al. (2004) suggested that MGI represents a novel class of agent which has functions in targeted genetic repair. Chee and Takami (2005) reported the presence of active MGI in the recA gene of *Geobacillus kaustophilus*. Pyle (2010) reported the structure and molecular data of MGI-based location of domain six (D-VI) and additional domain sites of the group IIA and IIB introns. Perutka et al. (2004) developed a computer-based algorithm to allow a swift and proficient disruption/simulation of bacterial gene. The algorithm drew target site recognition in limelight, and *E. coli* DExH/D-box protein and DNA helicase disruptants found in *Escherichia coli* were successfully analyzed in relationship to the function of these proteins. Further research on the role and applicability of MGIs in microorganisms, above all, on those thriving under extreme environments, will significantly contribute to our understanding on microbial functional diversity and its evolutionary significance.

3 Diversity of MGI

Numerous new MGIs are recorded in bacteria followed by archaea, in fungi and higher organisms (Fig. 1). More than 324 MGIs were detected in bacteria with ORF domain up to X (i.e., 10). Most of them were located on the chromosome, followed by the variety of plasmids, transposons, and integrons. As compared to bacteria, in archaea (few more than 16) MGIs were discovered. Most of them were located on the chromosome. The ORF-less MGIs also detected in bacteria and archaea were very few in number. All of them were located on the chromosome, followed by the plasmid. Similarly, remarkable numbers of intron fragments were found in bacteria as well as in archaea. All of them were located on chromosome followed by plasmid and transposons. Unlike prokaryotes, mobile group II introns were discovered on eukaryotes on mitochondrial DNA. Eukaryote such as liverwort, green plants, algae, fungi, yeasts, and *Ichthyospora* possesses mitochondrial MGI. Most of these were found in ORF domain RT 2-10 and RT-X. Liverworts, fungi, and yeasts possess MGI fused with upstream exons (Dai et al. 2003; Simon et al. 2008; Candales et al. 2012; Zimmerly 2014).

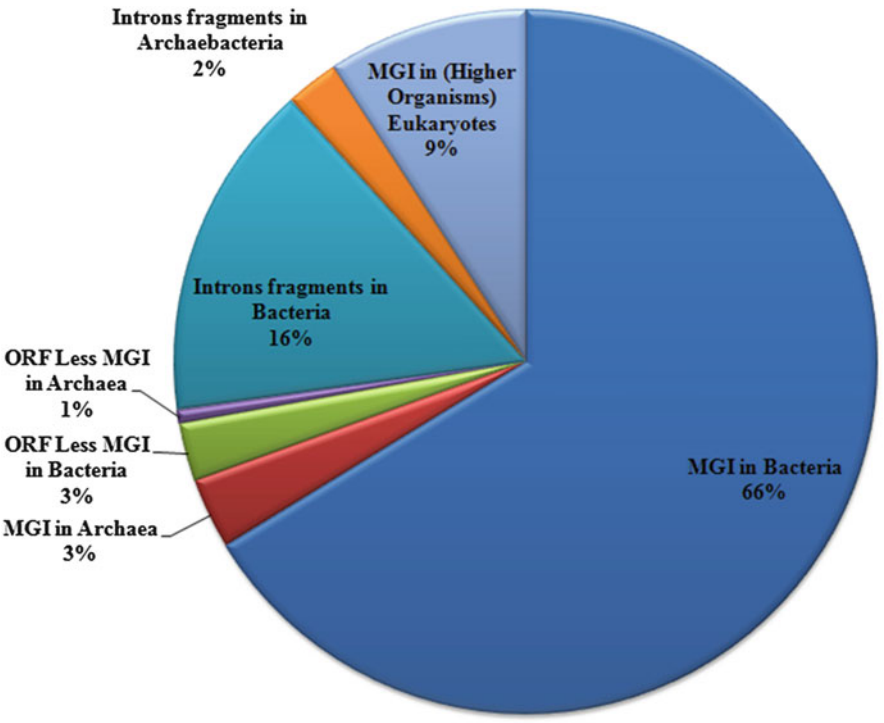

Fig. 1 Distribution and percentage of mobile group II introns among different life forms

4 Applications of MGI

Bacterial species are considered as dispersion-unlimited organisms due to its small dimensions and the broad range of metabolic capabilities. Thus, bacteria are able to reach and grow practically everywhere although the environment selects which species can develop under specific conditions (Prosser 2012). Joined to the huge bacterial diversity (Curtis et al. 2002) in our planet, the potential for developing under highly variable conditions turns into a surprising potential for bacterial growth on Earth and perhaps in other planets. Bacteria under optimum conditions, such as those provided in the laboratory, are able to show fast growth; however, scarce information is available on the functioning of bacteria in the environment and specifically under generally considered extreme conditions such as low or high temperatures (near 0 °C and up to 80 °C), low or high pH values, reduced water content, and high salinity, as the most typical examples occurring in nature. Seasonality is another cause of natural variability reaching extreme conditions above all as a result of current climate change events (Davidson and Janssens 2006; Rekadwad and Khobragade 2015, 2016). Genomes present a pool of all bacterial information available to thrive under easy and hardish conditions. However, our current understanding of the functional diversity of genes and the plasticity and flexibility of genomes is very limited. Recently, the diversity and abundance of tRNA genes have been suggested to be directly related to bacterial growth (Dana and Tuller 2014). Thus, growth and the events occurring on RNA and protein-processing mechanisms are essential to improve the understanding of functional capabilities and the genomic regulatory mechanisms of microbial metabolism and physiology (Table 1).

Thermophilic microorganisms including prokaryotes and eukaryotes are capable of growing under diverse and extreme environments such as high to low temperature, soil to water, acidic to alkaline, low-nutrient content, low water activity, etc. Base compositions in microorganisms are different and vary among species (Gomes and Steiner 2004). The genomic and physiological features developed by these microorganisms represent key adaptative mechanisms that are in need of further study in order to understand adaptation to extreme environments and their potential biotechnological application (Portillo et al. 2012; González et al. 2015; Santana and González, 2015). MGIs consist of a catalytically active intron RNA (ribozyme) and an intron-encoded protein (IEP). The combined activities of ribozyme and intron-encoded protein enable the proliferation of introns within genomes. The ribozyme (i.e., MGI-RNA) catalyzes its self-splicing through transesterification exactly similar that of spliceosomal introns, which yield spliced exons and an excised-intron-lariat RNA (LTR). The formed IEP is a multifunctional non-LTR-retrotransposon RT and related RT which assists splicing through stabilization of catalytically active RNA structure. It then remains a hurdle to LTR in a ribonucleoprotein (RNP) complex that invades DNA sites. DNA invasion caused ribozyme activity of intron (MGI-RNA), which reverses spliced into a host DNA strand. After invading host DNA strand, it's transcribed back into

Table 1 Spotlight on recently identified species having MGI

Species	MGI/enzyme	Ref.
Thermosynechococcus elongatus, *Geobacillus stearothermophilus*	Ribozyme	Mohr et al. (2013), Collins and Nilsen (2013)
Sinorhizobium meliloti, *Sinorhizobium medicae*	RmInt1	Toro et al. (2014)
Clostridium thermocellum	Catalytic RNAs, artificial nucleases, nucleic acid analogs, and peptide nucleic acids Xylanase, cellulase	Akinosho et al. (2014), Nakashima and Miyazaki (2014), Thomas et al. (2014)
Lactobacillus lactis	Ll.LtrB	
Podospora anserine	Possesses 15 group I introns and 1 MGI	Aguileta et al. (2014)
Coralline algae, *Gelidium vagum*, *Gelidium elegans*	MGI in the *chl*B gene	
Gracilaria chilensis, *Gracilaria salicornia*, *Gracilaria tenuistipitata* var. *liui*, *Grateloupia taiwanensis*	MGI in *trn*Me tRNA	Lee et al. (2016)
Liverwort	MGI	Hammani and Giege (2014)

new DNA by the IEP. Repeated cycles/invasion results in RNA splicing, and reverse splicing enables the invading introns to proliferate to new DNA sites and minimal impairing gene expression (Lambowitz and Zimmerly 2011). During the protein synthesis, each tRNA is changed and delivered into the ribosomes. Expression of tRNA genes has implications on the differential expression of different functional and structural proteins. Nowadays, the fast pace development in the field of transcriptomics and genomics has revealed structure and functions of many noncanonical tRNA genes. Disruption, fragmentation, rearrangement, minimization of tRNA and their re-coding, the relevance of non-coding RNAs, and a variety of small RNA sequences are included in these areas (Hartmann et al. 2004; Kanai 2013). At present, tRNA splicing is a boiling topic of hot debate because of their key role in the protein synthesis and influence in biological evolutions (Randau and Soll 2008; Di Giulio 2012).

5 Importance of MGI and Future Perspectives

MGIs are important elements present in microorganisms and organellar DNAs. Nevertheless, there is very limited information on their role and relevance in the adaptation of microorganisms to thrive under broadly variable environments and extreme conditions. Specifically, future perspectives on this topic will be focused on understanding the metabolic and physiological regulatory mechanisms

involving mobile group II introns, and this will be carried out in the base to the current large availability of massive sequencing datasets that have daily been added to public genomic (DNA) and transcriptomic (RNA) repositories.

Thermophiles have been reported to represent a highly diverse bacterial group showing extreme adaptability to broad ranges of conditions for different environmental factors. Their ubiquity and genomic diversity make them a major group of interest for biotechnology (including, e.g., the search for highly efficient and stable enzymes) and environmental bioremediation through biodegradation of recalcitrant pollutants. The biotechnological potential of this group is starting to be discovered, and it involves interdisciplinary perspective to understand their roles, their potentials, and their applications (Sakaff et al. 2012; Santana et al. 2013, 2015). Thermophilic MGIs have important roles in a variety of processes such as biorecuperation, bioremediation, and the biotechnological use of their enzymes under the highly diverse set of working conditions. Future research will clarify all these aspects and will contribute decisively to the development of our understanding of microbial evolution and physiology and their biotechnological potential.

The applications of the ongoing research and current knowledge of biotechnology are presumed to expand exponentially in the following years. An example (Fig. 2) is provided on a current application of mobile group II introns. Additional applications are about to be described, and the future opens a wide range of possibilities for the development of MGI-derived technologies. Future studies on MGI must be focused on gene-targeting vector (genetic tools)-based development. The bacterial MGI is independent upon recombination, which makes this

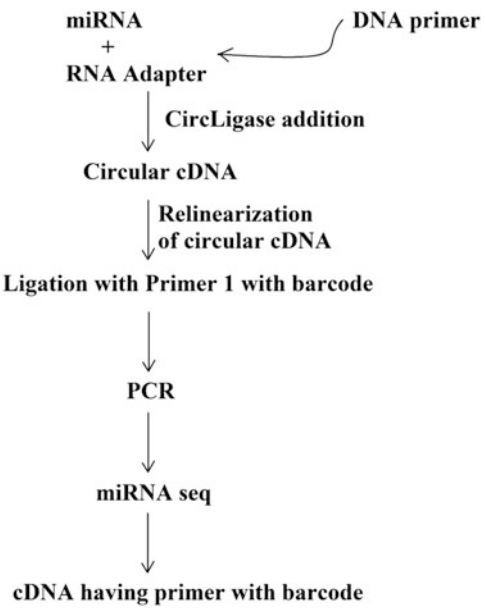

Fig. 2 Thermostable MGI reverse transcriptase (RT) (Enyeart et al. 2014)

technology broadly applicable. This extra feature of retroelements proved themselves as a potential tool for genetic manipulation in higher organisms (plants and animals) with lower chances of recombination. Additionally, certain host factors used by bacterial MGI contribute to intron-RNA folding and their mobility. In the near future, scientists should focus their research on the identification of factors (molecules) involved in increased functionality of bacterial MGI.

Studies on different aspects of MGI involving the architectural and functional organization are continuously helping us to understand the splicing mechanism of actively involved ribozymes and push us for parallel investigations on MGI and MGI-related elements such as retrotransposons and spliceosomes. Recently detected bacterial circular intron supports above statements that more study is needed to be performed in this particular area because MGI may be cosmopolitan in nature than we already thought. The mechanisms lying behind the splicing which occurred in MGI resulting in circle formation—formation of bacterial circular intron—and biological roles of such circles (circular introns) in MGI remain key issues expected to be resolved in the next few years.

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