Does the Promoter Constitute a Barrier in the Horizontal Transposon Transfer Process? Insight from *Bari* Transposons

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Abstract

The contribution of the transposons' promoter in the horizontal transfer process is quite overlooked in the scientific literature. To shed light on this aspect we have mimicked the horizontal transfer process in laboratory and assayed in a wide range of hosts (fly, human, yeast and bacteria) the promoter activity of the 5′ terminal sequences in *Bari1* and *Bari3*, two Drosophila transposons belonging to the *Tc1-mariner* superfamily. These sequences are able to drive the transcription of a reporter gene even in distantly related organisms at least at the episomal level. By combining bioinformatics and experimental approaches, we define two distinct promoter sequences for each terminal sequence analyzed, which allow transcriptional activity in prokaryotes and eukaryotes, respectively. We propose that the *Bari* family of transposons, and possibly other members of the *Tc1-mariner* superfamily, might have evolved "blurry promoters," which have facilitated their diffusion in many living organisms through horizontal transfer.

Key words: horizontal transposon transfer, promoter, Tc1-mariner, *Bari* transposons, promoter-luciferase assay.

Introduction

Horizontal gene transfer (HGT) is defined as the movement of genetic material between genomes not based on classic inheritance, i.e. parental generation to offspring passage of genetic material via sexual or asexual reproduction. HGT is well known to occur in prokaryotes, where 81% of their genes is estimated to be involved in HGT (Dagan et al. 2008). On the other hand only a limited number of genes has been observed to engage HGT in eukaryotes (Boto 2014). Although common on evolutionary time scale, HGT events occur too rarely and unpredictably to allow detailed studies in the laboratory (Houck et al. 1991; Gilbert et al. 2016). Therefore, there is an objective need for studying the biological features of the transferred sequences, where feasible, could be helpful in order to better understand the molecular characteristic explaining why some sequences undergo HGT more easily than others.

Mobile genetic elements or Transposable Elements (TEs) are discrete genomic fragments possessing an intrinsic ability to move and replicate within the genome. TEs are ubiquitous genome components, usually transmitted in a vertical way, from parents to offspring, however occasionally they spread across genomes of different species (Fortune et al. 2008; Ivancevic et al. 2013), a process known as Horizontal Transposon Transfer (HTT). Based on their physical structure

and their transposition mechanism, TEs can be classified into two main classes (Finnegan 1992). Class I elements, or retrotransposons, move via "copy and paste" mechanism in a way similar to the retroviral life cycle: a RNA intermediate is reverse transcribed into cDNA molecules, which are then inserted into the genome. Class II elements usually move via "cut and paste" mechanism in which the transposon is excised from one location and reintegrated elsewhere by means of a self-encoded transposase.

Besides their ability to replicate themselves within the genome, the evolutionary success of both Class I and Class II TEs largely depends on their ability to spread across species. Many Class I TEs share structural and functional similarity with retroviruses (Pelisson et al. 1997; Havecker et al. 2005), and for this reason they, intuitively, should be horizontally transferred more easily. However, this observation is in contrast with recent results from a systematic search for HTT in insects, which support the hypothesis that *Tc1/mariner* elements could be transferred more efficiently between distantly related taxa (Peccoud et al. 2017). Compared with DNA transposons, which do not usually require host factors for transposition (Plasterk et al. 1999), Class I elements may require several host factors for transposition that might be poorly conserved among taxa. These differences could explain the overall higher numbers of HTT events involving DNA transposons,

rather than retrotransposons, reported so far (Silva et al. 2004; Schaack et al. 2010; Peccoud et al. 2017).

Among the factors affecting the success of an HTT event, the initial expression of the enzymes necessary to perform the very first transposition event is expected to be crucial. This event would mostly rely on the promoter's ability to drive gene expression in a new genetic and genomic environment. In this regard Silva et al., suggested that "….in nature the integration and proper expression of a TE would seem to be a daunting impediment to successful horizontal transfer" (Silva et al. 2004). Despite its crucial importance, the promoter's role in HTT is, to the best of our knowledge, under-investigated in the scientific literature.

Theoretically, the transcriptional activation of TEs is possible both before and after the integration in the new genome, due to the presence of a promoter within the TE body. In particular, *Tc1*-like elements, belonging to Class II, can be transferred in an episomal form (i.e. covalently closed, extra-chromosomal, circular molecules), an intermediate of transposition observed both in eukaryotes (Radice and Emmons 1993) and prokaryotes (Polard and Chandler 1995). The transcriptional activation of TEs either as circular or linear molecules is absolutely required in order to survive in a totally unrelated genetic environment.

All autonomous TEs, both Class I and Class II, contain transcriptional regulatory regions that allow the transcription of the TE-encoded genes required to trigger transposition. Members of the *Ty3/Gypsy* and the *Ty1/copia* families are the best-studied Class I TEs, being widely diffused in all eukaryotes. They contain several types of transcriptional regulatory sequences (Cai and Levine 1995, 1997; Wilson et al. 1998; Minervini et al. 2010) that can also influence and consistently alter the expression of nearby genes (Peaston et al. 2004). By contrast, among Class-II TEs, members of the *Tc1-mariner* superfamily usually carry only a basal promoter, with few exceptions (Bire et al. 2016), without additional *cis*-regulatory sequences. The streamlined organization of the *Tc1-mariner* elements could facilitate the HTT process, under the hypothesis that the promoter of these elements could act as a regulatory sequence able to be recognized in distantly related genomic backgrounds.

In order to investigate if the promoter plays any role in the HTT success of *Tc1-mariner* elements, we used *Bari1* and *Bari3*, two Drosophila members of the superfamily as model elements.

The *Bari* family is composed of three subfamilies namely *Bari1*, *Bari2* and *Bari3*, identified in the vast majority of the Drosophila species (Moschetti et al. 1998; Palazzo et al. 2016). While *Bari2*-type elements are all inactive, both the *Bari1* and the *Bari3* subfamilies contain transposition-competent elements with a patchy distribution in geographically isolated Drosophila species (Palazzo et al. 2016). Consistent with the features of the *Tc1-mariner* superfamily, *Bari* transposons have a simple structure consisting of

Terminal Inverted Repeats (TIRs) at their ends surrounding a central sequence encoding the transposase. *Bari* elements contain three functional sequences, 18 nucleotides long, located within the 250 terminal nucleotides at each side, called Direct Repeats (DRs) (Plasterk et al. 1999) (Moschetti et al. 2008), responsible for the transposon-transposase interaction, a crucial step in the transposition event (Lampe et al. 1996; Vos et al. 1996). HTT events within the Drosophila genus have been inferred for some members of the *Bari* family (Dias and Carareto 2011; Palazzo et al. 2016; Wallau et al. 2016) supporting their ability to overcome the genetic barriers of the host species.

In this study we compared the promoter activity of *Bari1* and *Bari3* to *copia*, a Drosophila LTR-retrotransposon element that has a different evolutionary history and distribution in the Drosophila genus (Biemont and Cizeron 1999) and for which HTT events have been also reported (Bowen and McDonald 2001) (de Almeida and Carareto 2004). Our results suggest that the promoter does not constitute a barrier in the very early steps of the HTT process of the *Tc1-mariner* elements tested. Indeed, the promoters of *Bari* transposons display trans-Phylum, trans-Kingdom and trans-Domain ability to drive transcription, which represents an unprecedented feature of transposons' promoters. These results raise the question whether, and how, TEs have evolved flexible transcriptional regulation sequences that could facilitate horizontal transfers in new species, thus perpetuating themselves and escaping extinction.

Materials and Methods

Insect cells were cultured in Schneider's insect medium supplemented with 10% FBS, 1% penicillin/streptomycin, at 26 °C. Human cells were grown in Dulbecco's Minimum Essential Medium supplemented with 10% FBS, 200 mM glutamine, 1% penicillin/streptomycin, and maintained at 37 °C with 5% CO_2. iPSCs (line MS-C11, a generous gift of Dr Rosati) were generated from human skin biopsies and were grown in mTeSR™1 medium (StemCell Technologies Vancouver, Canada). *Saccharomyces cerevisiae*, strain BMA64-1A (MATa leu2-3,112 his3-11,15 trp1Δ ade2-1 ura3-1), was grown on YNB medium supplemented with the appropriate Dropout solution. *Escherichia coli*, strain DH5alpha, were grown on selective LB medium supplemented with antibiotics.

Transfections were performed in 6-well plates containing cells at 70% confluence using Translt LT1 (Mirus Bio, Madison, WI), and 1 µg of the appropriate plasmid or co-transfected with the Renilla luciferase construct (pRL-SV40; Promega, Madison, WI). The dual luciferase reporter assay system (Promega, Madison, WI) was used according to the manufacturer instructions. Both Firefly and Renilla luciferase activities measurements were recorded on GLOMAX 20/20 Luminometer (Promega, Madison, WI) 24-h posttransfection.
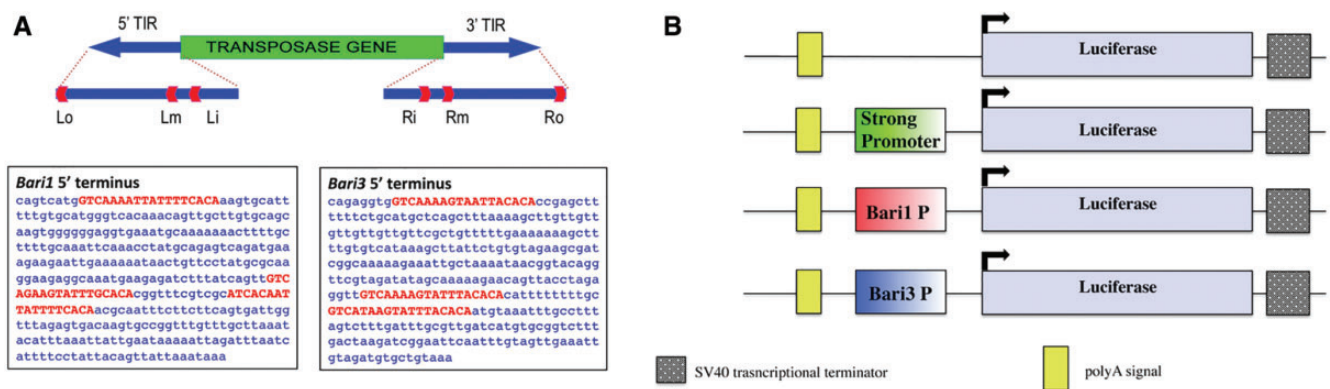
FIG. 1.—(A) General structure of the *Bari* transposons. The sequences tested in the promoter-luciferase assays are showed in the boxes. Position and sequences of the three DRs representing the transposase-binding sites, within the 5′ terminal sequences of both transposons are in red-boldfaced upper-cases. (B) Schematic structure of the reporter expression cassettes generated for this study. Arrows indicate the transcription direction.

Saccharomyces cerevisiae and *E. coli* transformants were assayed in the log phase. Yeast transformation was performed using the TRAFO methods described in (Gietz and Woods 2002). We have recorded three independent luciferase activity measurements per sample and the average value taken as sample measure. The average expression level from three replicates was normalized either to the average total protein content or to the Renilla luciferase measure to obtain the normalized luciferase activity taken as measure of the promoter activity. Statistical significance of the differences observed between the *Bari* promoters and the promoterless constructs was inferred using one tailed T-Student's test (H0: luciferase measurement not different between test and control).

A detailed procedure of plasmids construction is described in Supplementary Methods in the Supplementary Material online.

Positional weight matrices relative to the TATA, Inr and DPE core-promoter motifs were retrieved at YAPP Eukaryotic Core Promoter Predictor (www.bioinformatics.org/yapp/cgi-bin/yapp.cgi; last accessed March 2017). The PWM used are reported in the Supplementary Material online. Matrix scan analysis was performed using Regulatory Sequence Analysis Tools (RSAT) (http://rsat.sb-roscoff.fr/index.html; last accessed March 2017) (Medina-Rivera et al. 2015). Prokaryotic promoter predictions were performed using the BPROM tool (available at http://www.softberry.com; last accessed March 2017).

Results

Using conventional gene transfer methods we have mimicked HTT processes (see Materials and Methods) at four different scale levels, i.e. interspecies (*D. mojavensis* to *D. melanogaster*), inter-phyla (arthropoda to chordata, i.e. insect to human), inter-kingdom (Animalia to Fungi, i.e. fly to yeast) and inter-domain (Eukaryotes to Prokaryotes, i.e. fly to

bacteria). Assuming the absence of any other HTT-associated barriers (Silva et al. 2004) we have performed promoter-luciferase assays to determine the promoter activity of the terminal sequence located at 5′ of the *Bari* transposons in several cellular model systems. *Bari1* and *Bari3* were chosen as representative elements of the *Bari* families, due to their structural differences and because they are transpositionally active in natural populations, a necessary condition for a transposon to spread in a new genome through horizontal transfer. The assays were performed under transient reporter expression conditions in human and Drosophila cultured cell, while in *S. cerevisiae* and *E. coli* the assay was performed on selected clones stably expressing the reporter gene at the episomal level (see Material and Methods section).

The 5′ terminal sequences of *Bari1* and *Bari3* transposons assayed (hereafter called Ba1p and Ba3p, respectively) contain the binding sites for the respective transposase and are also supposed to contain the transposons' endogenous promoters (fig. 1A). The promoter-less luciferase cassette was used as a reference of the background expression. In addition, we used ad hoc positive controls, i.e. plasmids expressing luciferase under the control of a species-specific promoter, which also served as references to compare and quantify the *Bari*-derived promoters activity in each of the experimental system used in this study (fig. 1B).

The promoter activity of Ba1p and Ba3p was initially tested in *D. melanogaster* derivative S2R+ cells (fig. 2). As expected, the Ba1p sequence is able to drive the reporter transcription in cultured cell derived from the same host species. Also Ba3p sequence, which derives from *D. mojavensis*, (*D. mojavensis*–*D. melanogaster* divergence occurred ∼40 Ma; Tamura et al. 2004) supports the reporter transcription in S2R+ cells. Compared with the strong *copia* promoter of *D. melanogaster*, the promoter activities can be quantified as 25% and 11%, respectively, for Ba1p and Ba3p. Since the genome of *D. melanogaster* lacks *Bari3* elements (Palazzo et al. 2016), this result suggests that a hypothetical *Bari3* HTT event from

D. mojavensis to *D. melanogaster* would lead to its transposase expression.

In a hypothetical inter-phyla HTT event, we tested the promoter activity of Ba1p and Ba3p in three commonly used human cell lines and in undifferentiated induced pluripotent stem cells (iPSC). We found that both promoters were able to drive the luciferase transcription in HeLa, HepG2, Hek293 cells but not in iPSC.

As shown in figure 3, Ba1p and Ba3p always displayed a weak promoter activity that was nevertheless higher than a promoter-less vector. Compared with the viral SV40 promoter, which has slightly different activity in the three cell

Fig. 2.—The *Bari* promoters in *Drosophila* cells. The luciferase-promoter assays in S2R+ cells. The promoter activity is lower than the *copia* promoter (23% Ba1p and 15% Ba3p). Bars represent mean values. Circles represent actual data. **$P<0.005$.

lines tested, the activities of Ba1p and Ba3p are, respectively, 13% and 6% in HeLa cells, (fig. 3, left), 7,1% and 4,1% in Hek293 cells (fig. 3, middle) and 4,9% and 3,5% in HepG2 cells (fig. 3, right). Notably, the activity of the *D. melanogaster copia* promoter was not significantly different from the promoter-less construct (fig. 3, HeLa) highlighting that the low *Bari* promoters activity reflect a true activity of the transcriptional apparatus in mammalian cells.

We mimicked an inter-Kingdom HTT event using a laboratory *S. cerevisiae* strain transformed with our constructs. A new series of plasmids based on the yeast pFL39 vector were created including positive (pFL39/URA3p-luc) and negative (pFL39/luc) controls (see Materials and Methods section). We found a low but significant activity of the Ba1p promoter only, which represents roughly 5% of the yeast URA3 promoter activity (fig. 4). The absence of any detectable Ba3p promoter activity in *S. cerevisiae* suggests that the different efficiency of these Drosophila promoters in yeast could depend on the diversification in their structure and DNA sequence (see discussion).

Previous work has shown that trans-domain exchange of genetic material is also possible, involving transfers from bacteria to archaea and from prokaryotes to different types of eukaryotic cells such plants (Gelvin 2003), fungi (Heinemann and Sprague 1989; Inomata et al. 1994; Schroder et al. 2011) and human cells (Fernandez-Gonzalez et al. 2011). By contrast, DNA transfer from eukaryotes to prokaryotes is a rare event, apparently restricted to symbiotic or parasitic relationships (Keeling and Palmer 2008; Deschamps et al. 2014; Nikolaidis et al. 2014). Therefore, we tested Ba1p and Ba3p in *E. coli*, as a streamlined trans-domain HTT event in which studying the promoter activity. Unexpectedly, we found a strong luciferase activity in *E. coli* transformed with pGL3-
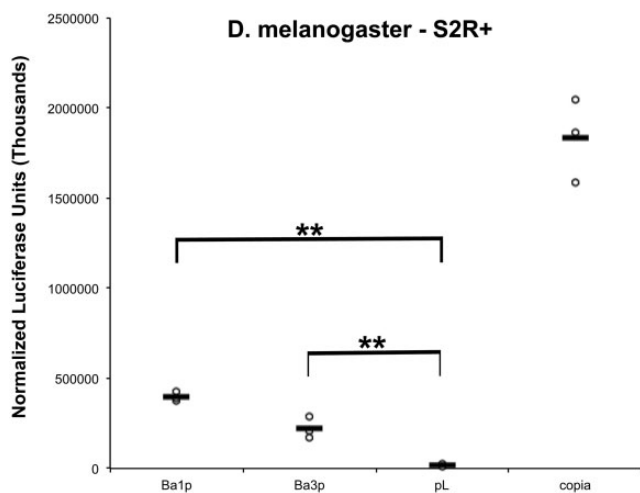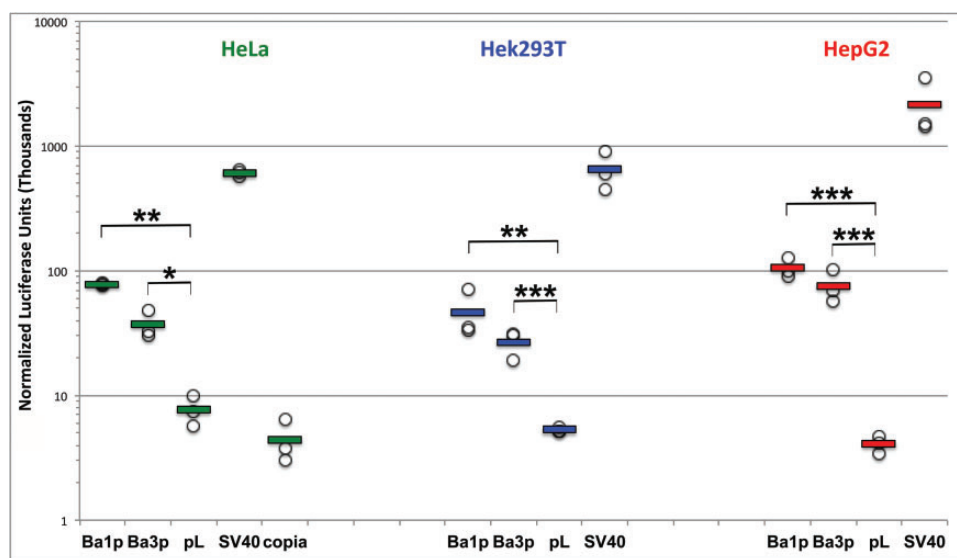

Fig. 3.—Promoter activity in human cells. Promoter-luciferase assays in HeLa cels (left, green), Hek293 cells (middle, blue), HepG2 cells (right, red). Bars represent mean values. Circles represent actual data. *$P<0.05$; **$P<0.005$; ***$P<0.001$. Note that the Y axis is in logarithmic scale.

Ba1p and pGL3-Ba3p constructs. Compared with the bacterial chloramphenicol acetyl-transferase promoter (CATp), the activity values represent 25% and 20%, respectively, for Ba1p and Ba3p (fig. 5A). Again, no promoter activity can be detected using the Drosophila strong *copia* promoter used as control suggesting a specificity of *Bari* transposon promoters in these heterologous expression systems (fig. 5A). Interestingly, the promoter activities of the Ba1p and Ba3p sequences are sufficiently strong to allow the direct observation of bioluminescence, after the addition of the luciferase

Fig. 4.—The *Bari* promoters in yeast. The Ba1p promoter activity is as much as the 5% of the URA3 promoter in *Saccharomyces cerevisiae*. The activity of Ba3p is not significantly different from the promoter-less construct. Bars represent mean values. Circles represent actual data. **$P<0.005$. Note that the Y axis is in logarithmic scale.

substrate in lysates obtained from cultures transfected with the pGL3/Ba1p and pGL3/Ba3p vectors (fig. 5B).

Given the transcriptional activation observed in a wide range of different organisms, we hypothesized that the sequences tested could contain multiple motifs recognized by TF binding sites in different species. Through a bioinformatics approach we predicted the presence of eukaryotic core-promoter motifs (i.e. TATA-box, InR and DPE motifs) in Ba1p and Ba3p (supplementary table 1, Supplementary Material online). Among many predicted motifs, Ba1p displays a TATA box (position 329) located 28 nucleotides upstream of an INR motif (position 357); this spacing is compatible with the previously described average spacing between these two motifs (Burke and Kadonaga 1997), suggesting the presence of a potential eukaryotic promoter in this site in the Ba1p sequence. In the Ba3p sequence one of the predicted TATA (position 50) is located 67 nucleotides upstream the adjacent INR motif (position 117), which exceeds the aforementioned average spacing, thus suggesting that this promoter prediction is weakly supported. It can be concluded that mapping the eukaryotic promoter in these sequences by mean of a simple bioinformatics analysis could be a difficult task. By contrast, a well-defined prokaryotic promoter can be predicted in both sequences (supplementary table 2, Supplementary Material online). In the Ba1p sequence the prokaryotic promoter is predicted downstream the DRs-containing region (-35 motif at position 287; -10 motif at position 307; TSS at position 322). In Ba3p sequence the predicted prokaryotic promoter overlap the innermost DR (-35 motif at position 235; -10 motif at position 257; TSS at position 272).

With the aim to map more precisely both the eukaryotic and prokaryotic putative promoters predicted in silico, we
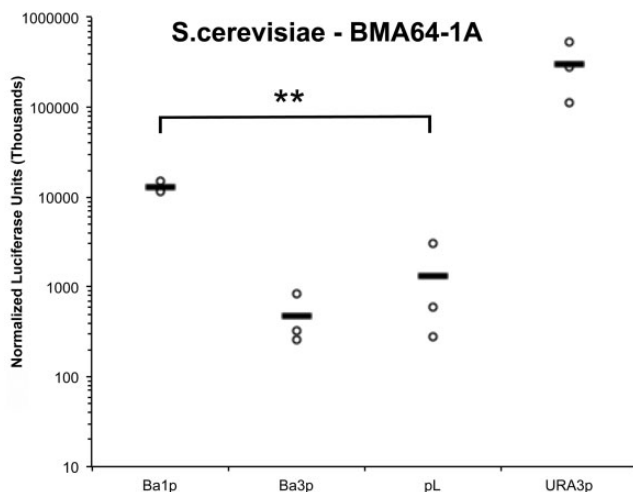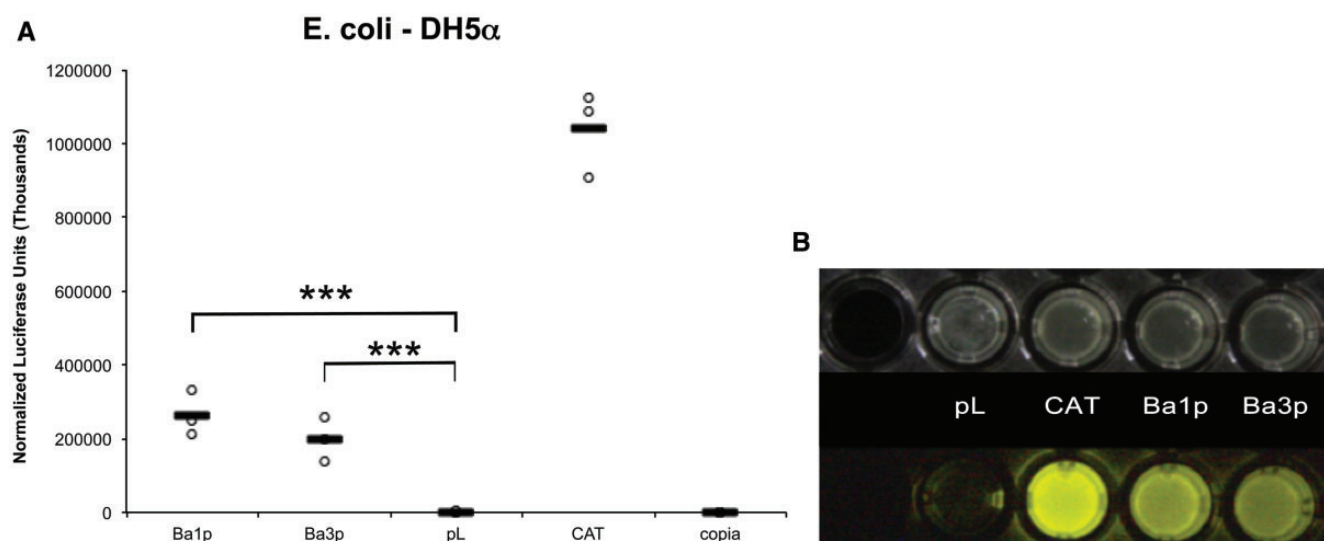

Fig. 5.—The activity of the *Bari* promoters in *Escherichia coli* can be estimated as the 25% and 20% (respectively for *Bari1* and for *Bari3*) compared with the CAT promoter activity (A). Direct visualization of the bioluminescence from transformed *E. coli* cultures in a dark room (B) when bacterial lysates are exposed to the luciferase substrate. Bars represent mean values. Circles represent actual data. ***$P<0.001$.
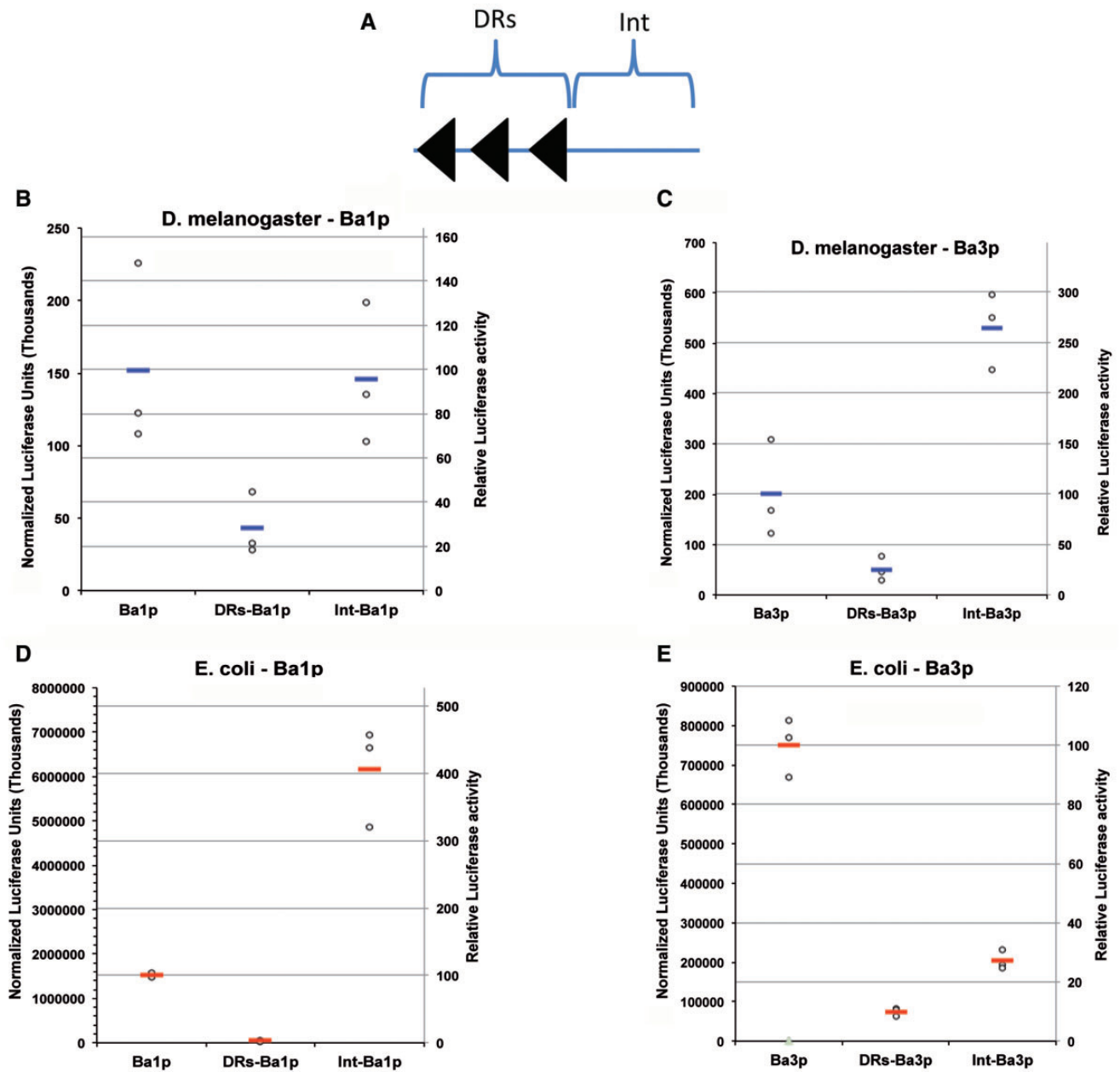
FIG. 6.—Mapping the eukaryotic and prokaryotic promoters in *Bari* transposons. The two sub-fragments analyzed are shown in (*A*). (*B, C*) Promoter activity in S2R+ cells of the two halves of the Ba1p and Ba3p sequences compared with the respective complete sequences. (*D, E*) Promoter activity in *Escherichia coli* cells using the two halves of the Ba1p and Ba3p sequences and the respective complete sequences. The relative promoter activity (compared with the respective complete sequence set to 100) is shown on the secondary *Y* axis.

generated additional constructs. Based on previous studies, which have mapped the eukaryotic promoter of *Tc1-mariner* elements in the intervening region between the inner DR and the translation start codon (Miskey et al. 2007; Walisko et al. 2008), we split Ba1p and Ba3p in two halves, the DRs-containing region and the intervening region ("DRs" and "Int," respectively, in fig. 6A), and tested them in promoter-luciferase assays in a eukaryotic (*D. melanogaster*) and a prokaryotic (*E. coli*) genetic context. The results in the

S2R+ cells clearly show that the Int region retains the promoter activity in both transposons (fig. 6B and C). Interestingly, the Int-Ba3p sequence displays a promoter activity greater than the parental sequence (i.e. Ba3p), suggesting that the DRs-containing region in the Ba3p sequence acts as a repressor in this insect cell line.

When we tested the DRs and Int regions in *E. coli* we observed that Int-Ba1p sequence retains the promoter activity, which is also higher, compared with the Ba1p activity,

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suggesting that the upstream sequence (DRs-Ba1p) acts as a prokaryotic transcriptional repressor (fig. 6D). We have also validated the Ba3p prokaryotic promoter prediction. The promoter activity is indeed mostly destroyed when the Ba3p sequence is split into two subsequences (fig. 6E), possibly due to the separation of the -10 and the -35 motifs, which have a critical role in the prokaryotic promoter function.

Discussion

The ability to drive transcription in many distantly related species' cells is an uncommon feature among promoters, although some TE-related examples have been reported (Ou-Lee et al. 1986; Copeland et al. 2007). To date, and to the best of our knowledge, a single viral promoter sequence is known to drive transcription in unrelated cells types. The 35S CaMV promoter is considered to be a strong constitutive plant promoter (Odell et al. 1985) and it also drives transgene transcription in plants cells outside the host range of the virus (Fromm et al. 1985), in *E. coli*, in budding yeast, in fission yeast and in higher vertebrates cells such as fish and mammalian cells [(Seternes et al. 2016) and references therein)]. Here we have unveiled that the endogenous promoters of two Drosophila transposons belonging to Class-II TEs are also able to drive transcription in a wide range of cells.

Bari1 and *Bari3* are related TEs of the *Tc1-mariner* superfamily with a nonoverlapping distribution in the Drosophila genus (Moschetti et al. 2008; Palazzo et al. 2016). The weak promoter activity of *Bari* elements observed in this work correlates well with the previously observed weak transposition activity (Palazzo et al. 2013), which could be further exacerbated by additional epigenetic control of the transposons (Specchia et al. 2010; Friedli and Trono 2015). Furthermore, many *Tc1-mariner* elements have low transcriptional activity (resulting from weak promoter activity) to limit the overexpression inhibition of the transposase (Hartl et al. 1997). These observations could justify the observed weak promoter activity of Ba1p and Ba3p.

Like HGT, HTT is a common phenomenon. However, we can detect only those events that have been successfully selected in the germline and for this what we see, in terms of frequency of HTT events, represents the tip of an iceberg. Horizontal transfer events can virtually occur in all direction and among all cell types (somatic, germline), posed that a vector is involved, or that the donor and receiving cells proximity allows the direct passage of genetic material. Here, we have observed that *Bari* promoters are functional in a number of different cell types and thus facilitating the overall HTT process.

Due to their presence in a limited sequence length, it could be questioned whether the presence of the promoter could be compatible with the usage of the transposase binding sites (DRs) within the left terminus of *Tc1*-like transposons. We have previously demonstrated that the transposase and the

RNA polymerase II activities only partially interfere with each other in both Ba1p and Ba3p (Palazzo et al. 2014), suggesting that the promoter and the DRs are sufficiently spaced apart.

Our results suggest that the eukaryotic promoter maps within the Int region of both *Bari1* and *Bari3* (122 bp and 100 bp, respectively), in agreement with the promoter position of *Sleeping Beauty* (SB) another well-studied *Tc1*-like transposons (Walisko et al. 2008).

The position of the prokaryotic promoter in Ba3p is more interesting for some aspects because it overlaps the rightmost DR in the *Bari3* left terminus. The inner DR has been demonstrated to be critical for the transposition of *SB* element and it could not be bounded by the transposase if the bacterial RNA polymerase occupies this site. However a dynamic exchange, depending on the binding kinetics of both enzymes to this region within the left terminus of *Bari3*, would still allow transposition in prokaryotic system.

Rather than a "universal core promoter," we propose that, *Bari* transposons and possibly other *Tc1-mariner* like elements contain "blurry promoters": AT-rich sequences containing several, weak core promoter motifs. This would allow for promoter recognition by TFs in distant species, overcoming the TFs divergence, and thus enable the promoter to drive transcription when it is transplanted in new genomes. *Bari*-derived promoters have these features indeed. However, these peculiarities could not grant a full recognition of these sequences as promoters in every cellular system, as suggested by the results in iPS cells and yeast (fig. 4). Blurry promoters could be one of the keys to understand why TEs are more susceptible to horizontal transfer in comparison with other coding and noncoding DNA sequences (Schaack et al. 2010). Besides their intrinsic mobile features, which allow them to enhance their horizontal movement potentiality and to increase their copy number per genome, a relaxed promoter, usually embedded in mobile elements, could facilitate the initial step of HTT. Finally, it is important to stress that the use of *copia* in our experiments has a double significance, since it is a strong Drosophila promoter isolated from a LTR retrotransposon. First, this is an example of the promoters' species-specificity, being the *copia* promoter activity not significantly different from the promoter-less construct in human and in *E. coli* cells (figs. 3 and 5A, respectively). Second, we speculate that, as hypothesized in the introduction, the promoter has a great positive impact for successful HTT of some Class II elements, facilitating their spreading. Our work might represent an early step towards the full understanding of the complex phenomenon of HTT. The role of transposon promoters and many other aspects, such as the destiny of an integrated transposon copy after the HTT event, especially in germline cells, are still to be deeply investigated in this view. Furthermore it would be interesting to extend this study to other *Tc1-mariner* like elements in order to establish how much common are blurry promoters in the superfamily.

Supplementary Material

Acknowledgments

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