BioMol Concepts, Vol. 2 (2011), pp. 333–341 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/BMC.2011.035

Review

Evolvability, epigenetics and transposable elements

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Abstract

Evolvability can be defined as the capacity of an individual to evolve and thus to capture adaptive mutations. Transposable elements (TE) are an important source of mutations in organisms. Their capacity to transpose within a genome, sometimes at a high rate, and their copy number regulation are environment-sensitive, as are the epigenetic pathways that mediate TE regulation in a genome. In this review we revisit the way we see evolvability with regard to transposable elements and epigenetics.

Keywords: environment; genome evolution; natural populations.

List of abbreviations

H-NS, histone-like nucleoid structuring protein; HSP, heat shock protein; IS, insertion sequence; LINE, long interspersed nuclear element; LTR, long terminal repeat; MITE, miniature inverted-repeat transposable element; rasiRNA, repeat-associated small interfering RNA; RNAi, RNA interference; SINE, small interspersed nuclear element; TE, transposable element.

Introduction

The last 10 years has seen a huge expansion of data about the sequences of genomes of different species and different populations. The field of epigenetics has also expanded considerably because this process is not only studied by molecular geneticists as evolutionary biologists have also started to think of epigenetics as something that could have an evolutionary impact. The new technologies for whole genome analyses were soon co-opted for analyzing epigenomes, yielding data about DNA methylation, chromatin modifications and even small RNA profiles across species and in some cases even across populations. The impact on genome regulation of epigenetic systems still tends to be ignored in classical population genetic models but this will clearly change in the near future (1, 2). The population variability of the epigenomes could soon be viewed as an important hallmark of the evolvability of species and populations.

Evolvability is related to the capacity of an organism to adapt to new environments, and it is now thought to be associated with its mutation capacity. A trade-off is expected between mutation load and increase in fitness. The idea is that the individuals in a population that are able to propose genetic novelties will be selected when confronted by new environments. These ideas are well documented in Prokaryotes, such as *Escherichia coli* but in Eukaryotes the experimental evidence is less abundant and more difficult to obtain. Like evolvability, which is associated with a changing environment, epigenetic marks are also known to be dependent on the environment. Evolvability and epigenetics could therefore be closely linked.

But how can we infer the ''mutability potential'' of a given species? Transposable elements (TEs) are an essential compartment of most genomes. These sequences sometimes occupy a large fraction of genomes and we are still trying to understand how genomes deal with their mutational ability. In recent years, it has become evident that TEs are targeted by epigenetic marks and that transposition regulation is mediated by epigenetic modifications (3). What also seems to be clear is that the targeting of a TE by an epigenetic mark (for example DNA methylation or a specific histone modification), sometimes spreads into nearby genes and thus modifies gene expression (4–6). Because TEs can insert randomly into a genome, we can expect that a large number of different combinations of gene expression will be available for selection at the population level. How this will be taken into account at the evolutionary level still needs to be determined. However, we can hypothesize that in a stable environment, specific combinations of TEs and chromatin structure will last long enough for neutral mutations to accumulate in the DNA sequence. On the other hand, if the environment is highly changeable, TEs and epigenetic marks can be modified giving rise to new networks.

In this review, we attempt to give an overview of recent advances in epigenetics related to TEs and of how environmental conditions can affect their relationship. We then go on to consider the relationship of epigenetics and TEs to evolvability.

Variability of the TE amounts in genomes

In the 1950s, studies of the DNA content of haploid genomes, also known as the C-value, in various organisms (7) revealed a paradox that remained baffling for many years: the C-value varies considerably between the different branches of the tree of life and is not correlated with the complexity of the organisms. Part of the solution to this par-

adox lies in the non-coding sequences of genomes and in particular in the moderately repeated fraction, which consists mainly of TEs.

First discovered in the 1950s by Barbara McClintock, TEs are sequences that can move and multiply along the chromosomes. They are found in the genomes of almost all living organisms, in proportions that are highly variable, even between species of the same taxonomic order (8). For instance, the recent sequencing of Insect genomes is a good illustration of this (Figure 1). The TE load ranges from no more than a few percent in the Honeybee genome (9) to almost half of the genomes of *Aedes aegypti* (10) and *Bombyx mori* (11). Among mosquitoes, the genome of *Culex quinquefasciatus* contains 29% of TEs (12), which is very different from its sister species, *Ae. aegypti*, with 47% TEs (10) and *Anopheles gambiae*, with no more than 16% TEs (13). Similarly, there is a threefold difference in TE content between *Drosophila melanogaster*, with a TE euchromatic content estimated to be 15%, and *Drosophila simulans* even though they diverged recently, only two to three million years ago (14). The recent comparative analysis of 12 *Drosophila* genomes revealed a considerable variation in TE content between these species, notably with a ten-fold difference between *D. simulans* and *Drosophila ananassae* (15).

Not only is the total load of TEs variable among genomes, but so too is the distribution of the different types of TE. Retrotransposons [TEs that are mobilized *via* an RNA intermediate; see Finnegan (16) for a classification of TEs] are commonly the preponderant class of TEs in Insect genomes, such as *D. melanogaster* (17), whereas almost all of the very few TEs that have been identified in the Honeybee genome are DNA transposons [TEs that are mobilized at the DNA level; see Finnegan (16) for a classification of TE] of the *mariner* family (9). The *Nasonia* TE repertoire is 10 times more diverse than that of the average dipteran (18). The major classes of TEs in the *B. mori* genome are LINEs and SINEs (comprising 14.5% and 13.3% of the genome, respectively), and LTR retrotransposons account for only 1.5% (10, 19), whereas LTR retrotransposons predominate in the TE repertoire of *D. melanogaster* (9). All taxonomic scales reveal such differences: in mosquitoes, *Ae. aegypti* and *An. gambiae* do not have exactly the same TE families [for instance, the *LOA*, *Osvaldo* and *Penelope* families are found in *Ae. aegypti* but not in *An. Gambiae*; (10)], nor are they present in the same abundances [*An. gambiae* displays 40-1340 MITE copies, whereas *Ae. aegypti* has 400-10 000; (20)]. The TE copy numbers in the different families vary greatly among mosquito species, whereas only minor differences are observed in *Drosophila* (21).

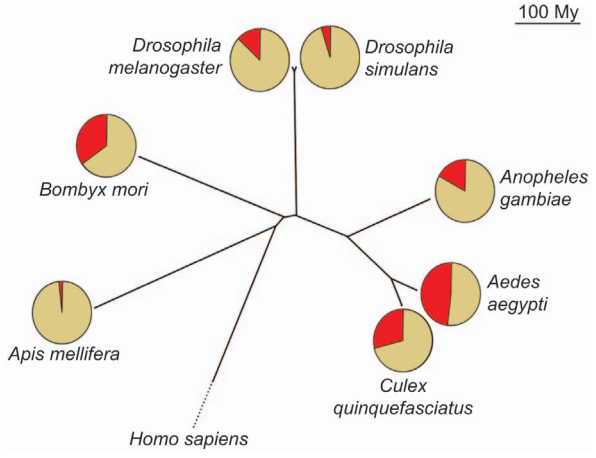

Figure 1 The frequencies of TEs in the genomes of a few well studied insect species are represented by the red sections. The relative abundances of TEs in these genomes are not correlated to the species phylogeny.

Such differences in TE content, even between closely related species, can arise as a result of various mechanisms or events. Differences in effective population sizes or in demographic histories have been proposed as possible causes in *D. melanogaster* and *D. simulans* (22, 23). The horizontal transfer of an active element from one species to another, as in the case of the P element of *D. melanogaster*, inherited from *Drosophila willistoni*, can drive the rapid evolution of a given genome (24). Differences in sex cycles are also proposed: one hypothesis to explain the paucity of retrotransposons observed in the Honeybee genome is that these sequences would be too detrimental in the haploid drones (9). We should also mention the effect of differences in genome structure and dynamics, which lead to differences in TE dynamics. For instance, the *B. mori* genome, which is 3.6 times larger than that of *D. melanogaster*, is filled with recently mobilized retrotransposons (25), whereas the TE copies identified in the *D. simulans* genome are relatively ancient and degraded (26).

In addition to species specific genomic characteristics, leading to diversity in TE loads in the different organisms, intra-species variability is also reported (27, 28). This is frequently linked to environmental heterogeneity and might correspond to a response to stressful conditions.

Interaction between TEs and the environment: activation of TEs following exposure to stressful conditions

Evidence of an increase in the number and mobility of TEs in response to stress has long been available, especially in bacteria, but the formal demonstration of a cause and effect relationship is relatively recent. Many studies have reported the mobilization or increase in copy number of bacterial Insertion Sequences (ISs, which are prokaryote specific TEs) following stressful conditions. Without attempting to be exhaustive, we can cite oxidative stress (29), high temperature (30), nutritional stress (31) and DNA damaging agents, such as UV light (32, 33) as such conditions. Drevinek et al. (29) provided direct evidence of the increase in TE activity in stressful environments by subjecting a strain with an inactive transposase to such conditions and observing that no IS movement occurred. Group II introns, which share a common ancestry with retrotransposons, are also mobile sequences found in Prokaryotes. It has also been shown that amino

acid starvation and oxidative stress induce mobilization in elements of this type (32).

Although there is abundant literature about prokaryotes in this field, probably because experimental evidence is easier to obtain, there are also some reports of TE activation under stressful conditions in eukaryotes. For instance in the case of nitrate starvation in marine diatoms (34), adenine starvation in yeast (35), heat shock in the fungus *Aspergillus oryzae* (36) and exposure to DNA-damaging agents, such as UV or gamma radiation (37). There have been a few reports of the effect of high temperature on TE activation in *Drosophila* but this remains controversial (38–40). Genomic stress, as in hybridization, has also been shown to have an impact on TE mobilization and activity in plants, mammals and insects (41).

On a global scale, Vieira et al. (27) describe a difference in the TE amount between natural populations of *D. melanogaster* and *D. simulans*, consisting of a gradient of the total TE amount between the African populations, in what is considered to be the cradle of the species, and the derived populations in Europe and the Americas. They propose that the observed increase in TE copy number of populations that have migrated out of Africa might be due to the stresses encountered by the colonizers (changes in temperature, humidity, food resources, pathogens etc.), which activate the TEs in their genomes. However, it is also possible to consider that the colonization of new environments is easier if the level of genetic variability and thus the level of evolvability is higher. We will return to this hypothesis below.

However, we should note that the literature on both Prokaryotes and Eukaryotes emphasizes the TE family specificity of the stress response. A given source of stress does not activate the entire load of TEs within a genome but only a subfraction, depending on the type of stress and the species considered. TE activation by stress does not seem to be a universal response of all TEs to all stressful conditions. For instance, Menees and Sandmeyer (42) report that the transposition of the yeast retrovirus-like element *Ty3* is inhibited under certain stressful conditions. We should beware of possible bias as a result of publication policy, which tends to favor positive results and therefore to underestimate the occurrence of a lack of effect, or indeed a negative effect, of stress on TEs (41). Nevertheless, it remains true that TE activation is a common phenotype in stressed cells and organisms.

Some molecular effectors have been identified as being responsible for the observed TE response to stress, such as the histone-like nucleoid structuring (H-NS) protein in Bacteria. This factor, the concentration and activity of which are sensitive to environmental changes, has been shown to promote the transposition of *Tn10* (43) and other bacterial elements (44). In Eukaryotes, a parallel may be drawn with what is known as epigenetic mechanisms and will be developed below. As recently discovered, TE regulation in Eukaryotes also depends mostly on the epigenetic machinery. Both the observed differences in total TE amounts between genomes and the TE activation reported in stressful conditions might therefore be related to differences in the epigenetic pathways.

Variability of TE regulation by epigenetic mechanisms

Epigenetic processes involve three major mechanisms: DNA methylation, histone modifications and RNA interference (RNAi), which interfere with the activity of genes as well as of TEs (3). Although TE regulation by epigenetic means is conserved among Eukaryotes, each of these three pathways relies on different molecular effectors, which differ depending on the organism and lead to differences in the efficiency of regulation. Regulation of TEs by DNA methylation has been proposed in *Drosophila* (45, 46) but still remains controversial (47). So far, the variability of DNA methylation patterns has been reported more frequently (48, 49) than the variability in the histone modification and rasiRNA (repeat-associated small interfering RNA) pathways, which have not often been investigated (50).

DNA methylation is generally considered to result in genetic silencing. It requires the action of DNA methyltransferases, three of which have been identified in vertebrates: Dnmt1, Dnmt2 and Dnmt3. Because the archetypal model Insect, *D. melanogaster,* like other Diptera that have been sequenced, has only one copy of the Dnmt2 gene (51, 52) it was thought that DNA methylation is spurious in Insects and might not be involved in TE regulation. However, studies of recently sequenced genomes have shown that Insects displaying all the functionally active, vertebrate-like DNA methyltransferases, including active CpG methyltransferases are not in fact so rare. For instance, *Apis mellifera* (8) and *Nasonia* species have three Dnmt1 genes; one Dnmt2 gene and one Dnmt3 gene (18). The stick insect *Medauroidea extradentata* also has a highly methylated genome, with methylated cytosines found within TEs and coding genes (53). In insects with these vertebrate like DNA methyltransferases, these genomes are methylated at CpG positions (9, 18), which is fairly infrequent in *Drosophila*.

Nevertheless, we do not end up with a dichotomy of either a vertebrate like or *Drosophila* like mode of DNA methylation. The Lepidopteran *Mamestra brassicae* presents a vertebrate like content of methylated cytosines but its TEs are not methylated (54). Krauss et al. (53) illustrate with the case of the walking stick *M. extradentata* that the amount of DNA methylation is inversely related to population size but positively correlated with cell turnover. *M. extradentata* is unusual in that it has small population sizes and high cell turnover during development. Not only does the quantity of methylated cytosines vary among Insect species but the roles of DNA methylation have also been shown to be diverse. Field et al. (55) review the methylation status in four insect species; *D. melanogaster*, *M. brassicae*, the aphid *Myzus persicae* and the coccid *Planococcus citri*, and reveal varying levels of methylation and functional diversity, ranging from a putative role in TE silencing to imprinting or gene regulation depending on the organism. DNA methylation might not even be associated with transcriptional silencing because some of the euchromatic regions transcribed in the aphid *Acyrthosiphon pisum* have been found to be highly methylated (56).

In a comparable fashion to the TE family specific response to stress that we have already mentioned, the use of the various epigenetic pathways also depends on the TE family. For instance, both the *gypsy* and *I* retrotransposons of *D. melanogaster* are regulated by rasiRNAs but with different effectors. *Gypsy* inhibition requires the somatic *flamenco* cascade, implying a chromosome region located in the euchromatin (57), whereas *I* is regulated by the germline regulation specific ping-pong pathway (58). It is therefore tempting to look for a parallel of this TE family specificity in both the response to stress and epigenetic regulation. Furthermore, in addition to the endogenous diversity between organisms in the effectors of the epigenetic regulatory pathways, variability also results from interaction with the environment. Indeed, epigenetic mechanisms have been shown to be very sensitive to environmental change.

Interaction between epigenetic TE regulation and the environment

Epigenetic mechanisms are highly sensitive to the environment. Gamma radiation has been shown to induce DNA hypomethylation in mammalian cell lines (59), as does exposure to heavy metals in plants (60). Maumus et al. (34) report a direct link between environmental stress and epigenetic regulation because they demonstrate that the *Blackbeard* element of the *Phaeodactylum tricornutum* marine diatom is hypomethylated in response to nitrate starvation. Similarly, reactive oxygen species, produced in the context of oxidative stress, alter the expression of genes involved in the DNA methylation machinery (61). Baccarelli et al. (62) have demonstrated that exposure to traffic particles induces hypomethylation of TEs in human blood cells. Servant et al. (63) have shown that the activation of the *Saccharomyces cerevisiae Ty1* element stressed by severe adenine starvation involves chromatin remodeling at the *Ty1* promoter. Genomic stress, such as inter-species hybridization in rice, has been shown to be associated with changes in the expression levels of genes involved in epigenetic mechanisms (DNA methylation and RNAi) and with extensive transgenerational alterations in cytosine methylation (64). Inter-species hybridization in Marsupials also leads to hypomethylation and TE amplification (65–67) but this does not seem to happen in all mammals (68).

Therefore, TE dependency on epigenetic machineries also leads to environmental sensitivity. Literature reports show a general trend that environmental modifications remove TE inhibition, which then allows TE mobilization.

TEs as major players in evolvability

The concept of evolvability has been increasingly developed and used in evolutionary biology (69). This concept is used in variable ways but here we will consider evolvability as the ability of an organism to adapt to new environments and thus the ability of a population of individuals to harbor sufficient genetic variability for new variants to be selected in changing environments. This could imply that selection for evolvability is correlated with selection for mutation, something that it is difficult to demonstrate experimentally, but advances have been made at the theoretical level (70, 71). In Prokaryotes, it has been shown that mutation is induced when cells are no longer adapted to their environment. As a result of this increase in the mutation rates, new variants that are better fitted to the environment can be selected (72). We can therefore suppose that the most evolvable organisms will be selected in a changing environment and we could expect evolvability to be reduced in a stable environment. In the light of this concept, we can try to integrate TEs as an important source of mutation. Because TEs are environment sensitive, as we have already shown, they may make a major contribution to the evolvability of organisms. Also, because TEs are regulated by epigenetic systems, which are also environment sensitive, it is appealing to try to integrate these different levels into the system and into the evolution of evolvability.

Stressful conditions generally modify the regulation of TEs, allowing them to be active, potentially leading to an increase in insertion site numbers. The increased mutation rate due to TE mobilization is a random process and so is not intended to produce beneficial changes. Even so, it can lead to adaptive changes more rapidly than regular mutation by means of substitutions or small indels. For instance, the insertion of a piece of retrotransposon upstream of the *Cyp6g1* gene of *D. melanogaster* provides the organism with insecticide resistance (73). TE mobilization can also trigger genomic rearrangements and lead to speciation events (41). However, even if the insertion of a TE is random, i.e., is equally likely to happen in any region of the genome, when a gene is the target of integration, insertion of TE tends to occur more frequently in the 5′ flanking sequences than in the coding or 3′ regions (74). This is presumably due to differences in chromatin accessibility, with the 5′ regulatory region of an active gene being less condensed than the rest of the sequence, allowing for transcription factor binding (75). Although TE insertions into non-coding DNA are likely to be neutral, insertions into the regulatory sequences of coding genes tend to affect the transcription level of the gene or its tempo of expression. Nevertheless, in any case, the consequences are assumed to be less deleterious than those of insertions that disrupt the coding sequence. These copies are therefore more likely to enhance the adaptive potential of the individuals carrying them. There is unsurprisingly a relatively abundant literature reporting cases of adaptive TE insertions located in the 5′ flanking sequences of coding genes (73, 74, 76–78).

Heat Shock Proteins (HSP) are produced particularly in a context of cellular stress and they act mainly as molecular chaperones (79). It is particularly interesting to note that the 5′ flanking regions of *Hsp* genes display some characteristics that make them particularly well suited targets for the insertion of certain TEs (75). In contrast, HSP90 has recently been shown to be involved in the suppression of TE mutagenic activity because these proteins are activated under

stressful conditions and can avoid mobilization (80). However, *Hsp*-directed insertions generally reduce *Hsp* transcript and protein amounts, and consequently reduce stress tolerance and decrease the inhibition of TE mobilization. In addition, TE insertions are targets for epigenetic regulation, and thus confer additional environmental sensitivity on the *Hsp* promoter. We can thus envisage that, although some of them may be detrimental, these TE insertions into the *Hsp* 5′ flanking regions will modify the stress sensor potential of the *Hsp* genes. As a result the organism becomes less tolerant to stress, which implies that *Hsps* are less efficient and are no longer able to prevent stress induced bursts of transposition. This situation allows new insertions to occur and some of them might be involved in adaptive changes (Figure 2). It should also be noted that because this variable sensitivity to environmental conditions relies on the epigenetic machinery it is reversible. In addition, the relationship between *Hsp* and epigenetics is even tighter because HSP90 interacts with the rasiRNA regulatory pathway (80) and molecularly associates with PIWI, a major actor of this machinery (81).

TEs thus appear to play a major part in genome evolvability. Their involvement is such that bacterial cells devoid of TEs are used in synthetic molecular applications in order to reduce their evolvability to a minimum and ensure that they continue to perform the function for which they have been designed (82, 83). In Insects, it is relevant to note that of the 12 *Drosophila* genomes to have been sequenced, the one with the lowest TE content is *D. grimshawi* (15), a Hawaiian endemic species that encounters a limited range of biotic and abiotic variations in its natural habitat. It is also known that the amount of TEs might vary at the population level. This has been clearly demonstrated for natural populations of *D. melanogaster* and *D. simulans*, as cited above. The differences in the amount of TE insertions are linked to the colonization history of the species and so we can speculate that the populations will have differing levels of genetic variability. It is tempting to think that the populations that were best able to colonize new environments were those with higher levels of genetic variability, which had more TEs and thus a higher degree of evolvability.

The evolvability of an organism is related to its capacity to adapt to new environments and it is now increasingly thought that selection might act on the evolvability of an individual. TEs confer a high level of evolvability on organisms because they are a source of genetic variability. Furthermore, because TE regulation is mediated by epigenetic factors, which are themselves environment-sensitive, this results in a considerable increase in the array of possibilities for facing new environments. Indeed, one of the bacteria with the most TEs, *Enterococcus faecalis*, in which more than 25% of the genome consists of mobile DNA, is also one of the most widely stress resistant prokaryotes (84).

Expert opinion

One of the major questions facing the biological sciences in the coming years will be how species are able to cope with new environments. The global environmental changes that have been observed are bound to impact on biodiversity but we still do not know about all the coping mechanisms that biological organisms have to enable them to face these new habitats. On the other hand, understanding how environments shape genomes is a real challenge. The epigenome analysis gives us some insights in how the phenotypes can be modulated but we still do not know how these systems will be transmitted in a permanent way. This questions will need further and deeper work. The place of repeated sequences

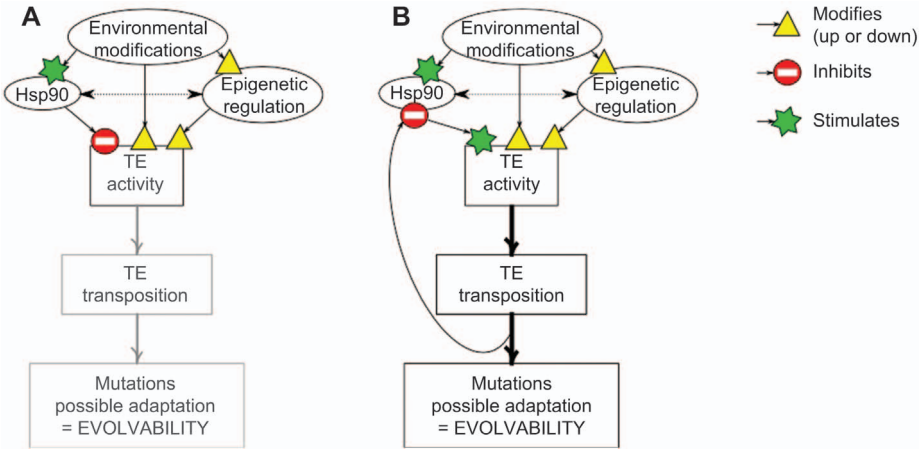

Figure 2 TE transcriptional activity can lead to TE transposition when all the players involved in the mobilization machinery are functional and not repressed.
(A) TE activity is regulated by the epigenetic machinery. Environmental changes, which are interpreted as stresses by organisms, can lead to a modification of the TE transcription level *via* modifications of the epigenetic pathway. In addition, under both standard and stressful conditions, TE activity, for being responsible for genomic instability, is inhibited by HSP90 (which also interacts closely with the RNA interference epigenetic machinery under standard conditions). (B) However, some TE copies might escape this silencing and preferentially insert into the *Hsp* promoter region, which then downregulates *Hsp* transcription and abolishes TE silencing. In a sort of amplification loop, TE mobilization can then take place, and adaptive mutational insertions might occur. This thus contributes to the evolvability of the genome.

such as TEs (which were long considered to be ''junk'' DNA) will also probably have to be reconsidered. Indeed, if TEs are the target of epigenetic regulation, they are also the target of environment and genetic novelty.

Outlook

We believe that more formal theoretical approaches will be developed in the future, as technical problems are overcome. Indeed, the analysis of repeated sequences is now facilitated by new sequencing methods, which will allow us to have enough sequences and be able to compare abundances. Also, the decreasing price of these techniques makes the analysis of populations feasible and will allow researchers to take into account different environmental conditions. The most important challenge will be to pool and integrate information obtained by molecular geneticists, ecologists and molecular evolutionists.

Acknowledgements

We would like to thank Christian Biémont for comments on this manuscript and Monika Ghosh for the English correction. The authors are funded by CNRS, ANR – 09-BLANC – 0103-01, FINOVI and CIBLE 2008 (Région Rhône Alpes).

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Received April 14, 2011; accepted July 11, 2011