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ORIGINAL PAPER

The significance of genome-wide transcriptional regulation in the evolution of stress tolerance

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Abstract It is widely recognized that stress plays an important role in directing the adaptive adjustment of an organism to changing environments. However, very little is known about the evolution of mechanisms that promote stress-induced variation. Adaptive transcriptional responses have been implicated in the evolution of tolerance to natural and anthropogenic stressors in the environment. Recent technological advances in transcriptomics provide a mechanistic understanding of biological pathways or processes involved in stress-induced phenotypic change. Furthermore, these studies are (semi) quantitative and provide insight into the reaction norms of identified target genes in response to specific stressors. We argue that plasticity in gene expression reaction norms may be important in the evolution of stress tolerance and adaptation to environmental stress. This review highlights the consequences of transcriptional plasticity of stress responses within a single generation and concludes that gene promoters containing a TATA box are more capable of rapid and variable responses than TATA-less genes. In addition, the consequences of plastic transcriptional responses to stress over multiple generations are discussed. Based on examples from the literature, we show that constitutive over expression of specific stress response genes results in stress adapted phenotypes. However, organisms with an innate capacity to buffer stress display plastic transcriptional responses. Finally, we call for an improved integration of the concept of phenotypic plasticity with studies that focus on the regulation of transcription.

Keywords TATA box · Cis-regulation · Microarray · Adaptation

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Introduction

Organisms cope with most adverse environmental conditions by inducing specific, and in some cases adaptive, stress responses. Phenotypic plasticity, in turn, is the ability of a genotype to modify phenotype expression in response to the environment (DeWitt and Scheiner 2004), which again can be adaptive, but also maladaptive or functionally neutral. Parsons (1987) postulated that phenotypic and genetic variations are increased in stressful environments (but see Schlichting and Pigliucci 1998, 1998 for a discussion), and that most of the stress-induced variation in quantitative traits is confined to a handful of loci. Transcription is a critical step in converting genotypes into phenotypes, although changes in transcript levels do not always modulate phenotype expression and visa versa (Wittkopp 2007). Complex phenotypic changes in response to environmental variables are frequently studied at (but also limited to) the transcriptional level. However, alternative splicing of mRNAs could provide another explanation for plasticity (Marden 2008), and has been shown to be the source of the impressive hypervariability in the innate immune surveillance molecules of the insects *Drosophila melanogaster* (Watson et al. 2005) and *Anopheles gambiae* (Dong et al. 2006). Since the development of transcriptome-wide gene expression analyses (transcriptomics), an overwhelming corpus of studies has demonstrated that abiotic stress is associated with differential expression of genes (Cossins et al. 2006; Roelofs et al. 2008). A major advantage of this approach is that these genes can be functionally analyzed to provide an understanding of the mechanistic basis of stress responses (Nota et al. 2008). If these effects can be studied in a biological system without genetic differences, the plasticity (reaction norm) of transcriptional change associated with physiological alterations caused by the environmental stress factor can be identified. It is thus highly appropriate to study the contribution of phenotypic plasticity to phenotypic evolution in the context of adaptation to abiotic stress, because phenotypic change (level of stress tolerance) and environmental stimulus (the stress factor) can be experimentally quantified together with associated transcriptional reaction norms. Plasticity, being subject to natural selection, will be favoured over fixed responses in organisms experiencing predictable environmental variations (David et al. 2004). In contrast, plasticity may reduce efficiency of natural selection, because it weakens consistency of the relationship between genotype and phenotype. Thus, plasticity may prevent a net change over time in order to maintain genetic and trait variation in populations. This review aims to highlight how plasticity in transcriptional response influences adaptation to abiotic stress by focusing on transcriptomic studies (microarray analyses) that have investigated the significance of genome-wide transcriptional regulation in genetic adaptation to stressful environments. Firstly, the concept of transcriptional regulation is introduced, followed by a discussion of the variation in transcriptional response associated with stress adaptation within a single generation. Secondly, we investigate the evolutionary consequences of transcriptional variation by reviewing studies that have encountered genetic adaptation to stress (at the transcriptional level) in natural populations exposed to stressful environments over multiple generations.

Transcriptional regulation

In this section we only highlight the major steps in the process of transcriptional regulation, for a full review on this topic we refer to Wray et al. (2003). This complex process is characterized by the integration of developmental, temporal, environmental, endocrine and

tissue specific signals, transmitted by the activation, nuclear translocation and binding of transcription factors to specific binding sites. Transcription factors (TF) are proteins that bind to specific DNA sequences upstream of the coding region of a gene (the promoter). The basal RNA polymerase II complex bound to an initiator or TATA box, can interact with TFs to determine the rate of transcriptional initiation: the frequency in a fixed time interval by which the RNA polymerase II complex leaves the initiator to transcribe the coding sequence into mRNA (Warren 2002). It, furthermore, involves a variety of interactions between proteins and DNA, including: (1) DNA looping, to bring together transcription factors bound to distantly positioned binding sites; (2) recruitment of co-factors i.e. proteins which do not bind DNA but modulate transcription by specific protein–protein interactions; and, (3) chromatin remodeling, an event that determines the accessibility of promoters for transcriptional complex binding. The resulting mRNAs are then processed (intron removal through splicing) and transported to ribosomes to be translated to functional proteins. It is important to note that the transcriptional activity of a gene is inducible through environmental cues spanning a large dynamic range.

Abiotic stress response

Physical factors (temperature, humidity, oxygen pressure, xenobiotic compounds) above a critical threshold, elicit a stress response. It is generally accepted that altered gene expression is one of the first signs of metabolic adjustment (Van Straalen and Roelofs 2006). The stress response at the molecular level can be dissected into three stages; sensing, signal transduction and transcription initiation. Kultz (2005) suggested that stress sensors monitor the degree of macromolecular integrity rather than an environmental signal, which would provide immediate feedback to activated stress response pathways. Lipid damage in membranes, misfolded intracellular proteins and DNA damage are the important macromolecular targets to sense stress. Additionally, increased reactive oxygen species (ROS) are an important second messenger for stress sensing (Mikkelsen and Wardman 2003). Heat stress is for instance sensed by high affinity binding of heat shock protein (HSP) 70 and HSP 83 to unfolding proteins, thereby releasing the transcriptional activator heat shock factor (HSF). Trimerization of HSF will induce transcription of hsp genes by binding to heat shock elements present in hsp promoters (Santoro 2000). The second step is the transduction of the stress signal towards the nucleus to evoke a transcriptional response, which is extremely complex in higher eukaryotes. A well known signaling pathway is the Stress Activated Protein Kinase Pathway. Upon conformational change due to the stress signal, either kinases or phosphatases can bind to (de) phosphorylated amino acid residues (a comprehensive overview is given by van Straalen and Roelofs 2006). This cascade typically terminates in the nuclear translocation and activation of a stress responsive transcription factor. Transcription is elicited and synthesis of gene products associated with stress commences. Finally, severe stress will ultimately lead to cell death via necrosis (pathological cell death) or apoptosis (programmed cell death, or cell suicide; Kultz 2005).

Transcriptional reaction norms and abiotic stress within a generation

The transciptome is dynamic and differential gene expression is essential for cell function, but also instrumental in facilitating flexible adaptation to fluctuating (abiotic) stress. To

maximize survival and reproduction (i.e. Darwinian fitness), it is therefore of paramount importance to fine tune the timing, efficiency and specificity of targeted responses (Gasch et al. 2000; Gracey et al. 2001; Owen et al. 2008). However, defining the precise boundaries of transcriptional norms is a major hurdle, mainly due to inter- and intra-species genomic differences. For instance, the metallothionein (*mt*) gene is efficiently regulated upon abiotic stress, such as heavy metals, to facilitate flexible adaptation to changing environments. In fact, the gene product has been shown to detoxify particular heavy metals by chelating free metal ions in the cell (Hensbergen et al. 1999; Roesijadi 1992). This MT-metal complex is in turn transported to vesicles and compartmentalized (Haq et al. 2003). Recently, Janssens et al. (2007) isolated several naturally occurring *mt* promoter (*pmt*) alleles from the soil arthropod *Orchesella cincta* and studied their inducibility upon cadmium- and oxidative stress with luciferase reporter assays. Surprisingly, basal expression levels of the alleles were significantly different. All *pmt* alleles were susceptible to Cd, but their reaction norms differed drastically, indicating that the variation in cis-regulatory units is important in driving the plasticity of gene expression. Metallothionein expression was also studied in detail in vivo, again showing pronounced differences between *pmt* alleles upon Cd challenge (Janssens 2008). Furthermore, a population genetic study showed that the frequency of the most inducible (plastic) pmt allele increased significantly with increased levels of soil metal pollution (Janssens et al. 2008). Recently, a microarray study (Roelofs et al. 2009) demonstrated that over 500 genes were significantly affected by metal pollution, and transcriptional responses were observed to be up to 100-fold up- or 20-fold down-regulated compared to the non-challenged control group. Closer inspection of the responses of specific functional categories revealed that effector genes (i.e. stress response genes, and genes involved in cuticle formation) showed a wider range of transcriptional activity compared both to housekeeping genes and genes involved in signaling. This observation has been independently confirmed by others (Denver et al. 2005).

Thermal stress has been studied extensively, also at the transcriptional level. A recent study of transcriptome changes in the liver of the eurythermal killifish, *Australofundulus limnaeus*, during thermal acclimation (Podrabsky and Somero 2004) has revealed a role for high mobility group b1 proteins (HMGB1) as general (i.e. not directly target gene-specific) activators of transcription. According to Podrabsky and Somero (2004), *HMGB1* mRNA content increases in response to a temperature decrease (either diurnal or seasonal) to effectively counteract the tendency of DNA to assume a more closed configuration at lower temperatures. HMGB1 promotes transcription, not in a global non-targeted fashion but by maintaining an 'open' DNA configuration of transcription factors and thereby allowing sequence-specific access to gene promoter regions. This mechanism is not inconsistent with the finding of a common expression footprint of 252 up-regulated genes in seven tissues of cold-acclimated common carp, *Cyprinus carpio* (Gracey et al. 2004). It would be intriguing to know whether an analogous mechanism underpins the specific suite of temperature-induced transcriptional changes, involving a number of energy metabolism genes observed in the soil-dwelling invertebrate *O. cincta* (Ellers et al. 2008). The HMGB1 response warrants further studies to ascertain its ubiquity as a modulator of acclimation to cold stress across a range of vertebrate and invertebrate taxa, and also to determine whether or not it facilitates transcriptomic adjustments in response to non-thermal stressors.

Although non-genomic model organisms facilitate an informative integration of ecologically-relevant traits, these studies are frequently hamstrung in terms of ascribing causality because of the unavailability of a full genome sequence and also the difficulty (if not impossibility) of performing genetic manipulations.

An ideal candidate and excellent investigative tool that meets the challenge of determining the timing efficiency, and specificity of gene regulation in response to stressors is the nematode worm *Caenorhabditis elegans*. Due to its self-fertilizing propagation, which results in offspring that contain a genome architecture that is essentially clonal, the genetic variability that inevitably accompanies sexual reproduction is largely eliminated. Transcriptional responses are nevertheless highly plastic in *C. elegans*. Some compounds, such as toxic levels of heavy metals (Calafato et al. 2008; Swain et al. 2004), can reduce *C. elegans* survival rates; others, for example quercetin, extend life-span (Pietsch et al. 2009). Such responses have, moreover, been linked to specific genes and pathways. Under field conditions abiotic stress is, however, seldom uni-dimensional, thus the task of defining transcriptional norms and deciphering molecular genetic response pathways is considerably more challenging (Steinberg et al. 2008), although not impossible as demonstrated by studies on humic substances (Menzel et al. 2005) and river sediment mixture toxicity (Menzel et al. 2009).

A striking response to abiotic stress in *C. elegans* is the transformation of larvae into facultative dauers. The existence of a cryptobiotic stage is, of course, an evolutionary strategy that is not restricted to nematodes. Other examples include the gemmules in sponges or tuns in tardigrades, both of which ensure survival during harsh environmental conditions. In nematodes, cryptobiotic dauer larvae are resistant to stress, they stop feeding and arrest in development, and they are able to survive for months rather than weeks (Cassada and Russell 1975). Unfavorable stimuli (e.g. lack of food, stress or crowding) induce the transition from the L1 larval stage to the dauer physiological state, a process that is driven by a pheromone that targets the TGFbeta, cGMP and insulin-like signalling pathways (Birnby et al. 2000; Kimura et al. 1997; Ren et al. 1996). The underlying genetics of dauer formation and the molecular basis of plasticity are of course more complex (see the seminal review by Fielenbach and Antebi 2008 for details) and are modulated by global changes in transcription levels. In general, transcription is depressed by 11–17% during dauer but rapidly increases upon dauer termination and development to the L4 stage (Dalley and Golomb 1992). However, the relative expression changes of individual genes are remarkable, with about 10% of the transcriptome (1,984 genes) significantly dauer-regulated, including specific genes that are induced early in dauer, others that are induced immediately before 'release' from dauer into L4, and yet others whose induction increases continuously throughout the dauer phase (Wang and Kim 2003). Although dauer formation can prolong the lifespan of a nematode by a factor of 5, post-dauer aging is normal (Klass and Hirsh 1976).

Caenorhabditis briggsae, a related nematode species, seems to have a homologous dauer pathway, with evolutionary conserved dauer orthologs. Like *C. elegans*, it enters dauer in response to unfavorable environmental conditions; however, the temperature tolerance of *C. briggsae* seems to be several degrees higher than that of *C. elegans* (Inoue et al. 2007), which has been shown to correlate with the occupation of different ecological niches within overlapping geographic locations (Cutter et al. 2006). Overall, this emphasizes that the transcriptional norm is defined by the need to respond to environmental change and maintain phenotypic plasticity, demonstrating the ability of one genotype to engender different phenotypes in response to environmental challenge (Lopez-Maury et al. 2008).

All, but a few, laboratories use the same cryopreserved strain of *C. elegans*, namely an isolate from Bristol, UK. However, *C. elegans* is a globally omnipresent species, and due to inevitable geographical isolation over time, natural genetic variation is apparently reflected by numerous single nucleotide polymorphisms (SNPs), deletions and insertions.

The most divergent wild type strains are N2 and CB4856 with a SNP, on average, every 840 bp (Swan et al. 2002) and some 500 deletions throughout the genome (Dalley and Golomb 1992). This feature has been exploited in genomics-based studies, where quantitative trait loci (QTL) mapping is capable of pinpointing the master molecular-genetic regulators driving plasticity. One notable example by Li et al. (2006) identifies the plasticity of gene expression in *C. elegans* exposed to different temperatures. Interestingly, differential gene expression has been shown to be enriched for specific functional categories, chromosomal locations and coexpression in evolutionary divergent isolates, demonstrating that selective forces shape local expression patterns (Denver et al. 2005; Jordan et al. 2008). Although the divergence of *C. elegans* strains is markedly lower compared to sexually reproducing species, it demonstrates that miniscule genetic 'tweaks' can result in pronounced changes in phenotypic plasticity. Indeed, the complexity of a phenotype is the consequence of the interaction between genes and the environment.

Transcriptional regulatory evolution and stress tolerance

The evolutionary process of adaptive transcriptional regulation in a clearly-defined ecological framework has been substantiated in a number of cases throughout different life forms. Environmental stress is a selection pressure to which organisms have adapted under prolonged exposure, resulting in tolerant/resistant natural populations. Transcriptome-wide analyses have recently shed more light on the role of transcriptional regulation in stress adaptation. Insecticide resistance represents an important example of natural selection. Daborn et al. (2002) studied the transcriptional regulation of the DDT-R locus on chromosome 2 of *D. melanogaster*, a locus that confers resistance not only to DDT, but also shows cross-resistance to a number of existing and novel insecticides. The DDT-R locus is associated with the cytochrome P450 gene cyp6G1. Using DDT-R strains, Daborn et al. (2002) were able to identify a severe over-transcription of cyp6G1 compared to >90 other cyp450 genes. The over-transcription of cyp6G1 could be localized to an insertion of an Accord transposon into the 5′ untranslated region of the gene. This suggests that cyp6G1 lost its plastic response due to insecticide selection pressure. Remarkably, this (molecular) phenotype spread globally within decades reaching almost fixation in non-African *Drosophila* populations (Catania et al. 2004).

The over-expression of target stress response genes as a mechanism of adaptive evolution of stress tolerance have also been identified in other invertebrate taxa. For example, metal tolerant populations of *O. cincta* are linked to the constitutive over expression of the metallothionein gene (Roelofs et al. 2007; Sterenborg 2003). A specific *mt* promoter allele was shown to be increased in *O. cincta* populations inhabiting environments with elevated metal concentrations (Janssens et al. 2008). This allele displayed the steepest reaction norm among the tested *pmt* alleles (Janssens et al. 2007), as well as the highest maximum induction potential upon cadmium challenge. In the adapted populations, however, this allele was constitutively expressed such that induction by cadmium was negligible. The inducibility (plasticity) was seemingly lost in favour of adaptive evolution of metal tolerance, implying that factors in trans (genetic background) may also be targets of natural selection. In a recent microarray study, cadmium-induced gene expression of a tolerant population was compared to a metal sensitive reference population (Roelofs et al. 2009). Clear differences between the two strains/ecotypes at the transcriptional level were observed. The reference population showed an unequivocal signature of stress-induced perturbation of gene expression in response to the heavy metal challenge (Fig. 1a), that

was significantly different to the tolerant population which lost part of its plastic response (Fig. 1b). Gene ontology analysis revealed that the genes are functionally implicated in cuticle formation, stress response and chromatin remodeling. The data suggest that abiotic stress may be able to drive evolution through constitutive over-expression of genetic stress response networks. The regulatory hubs that control these networks may be the key to explain adaptive phenotypes. Previous studies have provided evidence that whilst metal adapted populations matured earlier and juvenile body growth was faster, the adapted phenotype did not result in reduced fecundity (Posthuma et al. 1992, 1993). Stress adaptation through transcriptional regulatory evolution has also been implicated in humans. A very elegant study by Tishkoff et al. (2007) provided clear evidence of convergent evolution of lactose tolerance by enhanced transcription of the lactase gene (*LCT*) in adult

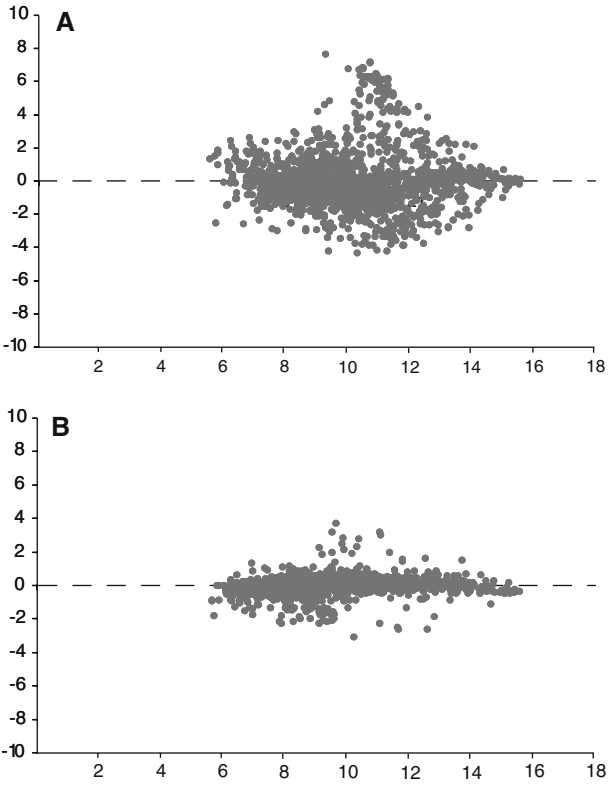

Fig. 1 Transcriptional reaction norms from a microarray study with cadmium (Cd) challenged *Orchesella cincta* populations. MA plot of two populations; **a** Reference strain from a population sampled at a clean site; **b** tolerant strain derived from a population sampled at a heavy metal (cadmium, lead and zinc) polluted abandoned mining site. A strong signature of stress-induced perturbation of gene expression in response to Cd is observed in the reference strain (^{2}Log differential expression ratio's range between -4 and $+8$; Fig. 1a), while the tolerant animals lost most of this stress-induced gene expression signature (e.g. strongly diminished maximum up regulation of (^{2}Log differential expression ratio's range between -3 and $+4$, Fig. 1b). *x*-axis mean ^{2}Log total hybridization intensity of control and Cd exposed animals ($M = (^2\text{Log control} + {}^2\text{Log Cd treatment})/2$); *y*-axis Log_2 ratio Cd treated vs control animals ($A = {}^2\text{Log Cd treatment} - {}^2\text{Log control}$). Hence, cDNA probes with positive *y* values represent genes that are upregulated due to Cd treatment, while cDNA probes with negative *y* values represent genes that are down regulated due to Cd treatment. A total of 1,600 cDNAs were present on the microarray

Africans and Europeans. This gene is essential for the digestion of lactose, the main carbohydrate in milk, into sugars. However, activity declines rapidly after weaning due to decreasing *LCT* enzyme levels. Derived cys-regulatory elements that increase *LCT* transcription caused a selective sweep over the past 7,000 years in Northern Europeans and pastoralist populations from Africa. Convergent evolution was driven by a strong selection pressure as a consequence of shared animal domestication and adult milk consumption. Another example on stress adaptation through transcriptional regulatory evolution in humans was very recently published by Luca et al. (2009). The serum glucocorticoid-regulated kinase 1 (SGK1) promotes cellular homeostasis in response to stress. Luca et al. (2009) showed that human populations living nearby the equator have increased glucocorticoid receptor mediated SGK1 transcription resulting in increased stress response. It was suggested that variation in SGK1 transcriptional regulation could favor negative effects of glucocorticoid mediated stress response predisposing individuals to chronic diseases such as hypertension. Adaptive evolution of stress tolerance associated with constitutive over-expression was also shown in the metal hyperaccumuating plant *Thlaspi caerulescens* (van de Mortel et al. 2006). Besides stress response genes (metallothioneins), genes involved in metal homeostasis (Zn transporters) and lignin biosynthesis were constitutively over-expressed. Finally, up regulation of error-prone DNA polymerase, resulting in increased mutagenesis and evolvability, has been associated with stress-adapted evolution in bacteria (Foster 2005).

Environmental stress as driver of adaptive evolution through increased transcription of stress response genes should, however, not be treated as a general, universally applicable, concept. If increased constitutive transcription of stress response genes drives adaptive evolution of stress tolerance, we predict over- expression of hsp70 to be a key factor in high temperature adapted *Drosophila*. Surprisingly, several independent selection studies identified the presence of lower hsp70 expression in adapted phenotypes and other physiological processes were the target of selection (Sorensen and Loeschcke 2002). Furthermore, absence of adaptive evolution of transcription regulation, despite the presence of a clear abiotic selection pressure, has also been reported. This was exemplified in the rufous-collard sparrow (*Zonotrichia capensis*) distributed along an altitudinal gradient (Cheviron et al. 2008). Cold and hypoxic conditions interact to create a stressful environment in which energy metabolism needs to be maintained in order to adapt to low oxygen availability and thermal stress. It is well known that natural selection can mediate adaptive evolution in certain vertebrates to create adaptive phenotypes at high altitudes (Frisancho 1975). These studies have recently been supported by evidence of adaptive divergence (polymorphism in mtDNA) at the molecular level (Ruiz-Pesini et al. 2004), although the adaptive role of transcriptional variation in high-altitude environments was not addressed. However, the study by Cheviron et al. (2008) yielded contrasting results. They studied transcriptomic profiles from the muscle tissue of the rufous-collard sparrow native along an altitudinal gradient in the Pacific Andes ranging from 2,000 to 4,100 m above sea level. Significant differences in regulation of transcripts involved in oxidative phosphorylation, oxidative stress response, protein synthesis and signal transduction were observed. To assess plasticity in these differences a common garden experiment at 150 m above sea level was conducted and, interestingly, none of the differentially expressed transcripts remained significant. The study demonstrates that cold- and hypoxia-tolerance in rufous-collared sparrows is highly plastic. Consequently, the high level of transcriptional plasticity prevented the adaptive evolution of cold/hypoxia tolerant phenotype. This is of particular interest, since gene flow along the studied altitudinal gradient is substantially reduced, suggesting that there is scope for adaptive divergence. In any case, the above

mentioned studies exemplify the difficulty to predict which strategy is actually favored by evolution.

Stress-induced transcription and the TATA box

As noted earlier, most protein-coding genes are transcribed by RNA polymerase II. Transcriptional initiation requires assembly of this polymerase to a core promoter structure such as the TATA box which is present in about 20% of *Saccharomyces cerevisiae* genes (Basehoar et al. 2004). Remarkably, TATA-containing genes seem to be enriched in stress response genes, a notion that applies to plants (Walther et al. 2007) and humans (Yang et al. 2007), and suggests that TATA motifs are associated with rapid and variable regulation. Indeed, it was shown that stress-related proteins expressed from genes with a TATA box exhibit a high level of intrinsic variability (Newman et al. 2006). Moreover, Blake et al. (2006) showed that this intrinsic variability enables rapid individual cell responses which results in a 'burst' of gene expression that confers a clear benefit when facing acute environmental stress. In other words, the TATA box increases the plasticity of gene regulation and is associated with stress response genes. In contrast, TATA-less genes are enriched among housekeeping genes and growth-related genes (Basehoar et al. 2004). This suggests that transcriptional control may be bipolar with distinct genetic elements in core promoters regulating either growth- or stress related genes (Lopez-Maury et al. 2008).

Evolution of stress tolerance and the TATA box

Since stress response genes are enriched in TATA containing genes, it might be expected that TATA boxes show rapid regulatory evolution specifically when abiotic stress is the selective force. This hypothesis was tested by Tirosh et al. (2006) in a systems biology context. The study provided clear evidence that long-term adaptive evolution of stress tolerance among yeast species is correlated with short term regulatory changes to environmental stress. The study also showed that stress response genes with TATA box show exceptional rapid regulatory evolution, not only in yeast but also in *C. elegans*, fruit fly, plants and mammals (Tirosh et al. 2006). Furthermore, experimental mutation accumulation studies show a significant correlation between transcriptional plasticity and mutation variance in TATA box containing stress response genes (Landry et al. 2007). Taken together, these data highlight the importance of the TATA box containing stress response genes in both short- and long-term regulatory adaptation.

Conclusion and perspective

In order to stay competitive, organisms need to balance reproduction and the protection against environmental stress. Transcriptional plasticity supports survival in variable stressful environments, at least in the short term. When a particular stress factor persists over multiple generations, adaptation may result in persistent changes in gene expression, namely constitutive over expression of specific stress response genes, resulting in an adaptive phenotype. The stress-adapted transcriptional profile suggests an elevated but decreased flexibility in transcriptional responses to cope with the selective agent. Still, it is difficult to predict such an evolutionary scenario, because few studies show

contrasting results (down regulation of stress response genes in stress adapted phenotypes). Moreover, we discussed evidence from the literature where genome-wide transcriptional plasticity seems to counteract adaptive evolution towards a stress tolerant phenotype. However, the role of genome-wide transcriptional responses in stress acclimation and adaptive evolution of stress tolerance are still poorly understood, although there is recognition that phenotypes emanating from plasticity can become stabilized via genetic assimilation as less optimal pathways are closed down (Schlichting and Smith 2002).

Data from systems biology studies on genomic model organisms imply that the TATA box, a cis-regulatory genetic element, is overrepresented in stress response genes. Remarkably, TATA box-containing stress response genes show an increased transcriptional divergence and it could be argued that TATA boxes may foster the modification of gene expression characteristics. Indeed, experimental data provided evidence that mutations in and around a TATA box change the plasticity of gene expression (Blake et al. 2006). Although the use of well established genetic/genomic model organisms play an essential role in obtaining mechanistic evidence, a draw back has to be their lack of ecological relevance. In any case, the recent technological improvements (high throughput sequencing and qRT-PCR, etc.) will trigger a new generation of hypotheses testing, namely genomic studies derived from ecologically relevant organisms currently with limited genomic information. For instance, the genome of *Lumbricus rubellus* is currently being elucidated with the aid of massively parallel sequencing (Sturzenbaum et al. 2009). With this information in hand, it will, for example, be feasible to test whether the adaptation of *L. rubellus* to Copper contamination was driven by evolutionary divergence and selection on TATA box-containing genes. These developments will increase our knowledge base on the mechanistic role of transcriptional plasticity to cope with changing environments and to what extent this drives adaptive evolution of stress tolerance.

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