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INVITED REVIEW

The Effect of Stress on Genome Regulation and Structure

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• *Background* Stresses exert evolutionary pressures on all organisms, which have developed sophisticated responses to cope and survive. These responses involve cellular physiology, gene regulation and genome remodelling.
• *Scope* In this review, the effects of stress on genomes and the connected responses are considered. Recent developments in our understanding of epigenetic genome regulation, including the role of RNA interference (RNAi), suggest a function for this in stress initiation and response. We review our knowledge of how different stresses, tissue culture, pathogen attack, abiotic stress, and hybridization, affect genomes. Using allopolyploid hybridization as an example, we examine mechanisms that may mediate genomic responses, focusing on RNAi-mediated perturbations.
• *Conclusions* A common response to stresses may be the relaxation of epigenetic regulation, leading to activation of suppressed sequences and secondary effects as regulatory systems attempt to re-establish genomic order.

Key words: Genomic shock, stress, pathogen, genome, hybridization, allopolyploid, transposon, heterochromatin.

INTRODUCTION

Stress, in any form, exerts strong evolutionary pressure on all organisms. To survive, any organism must develop tolerance, resistance or avoidance mechanisms. Tolerance allows the organism to withstand the assault unharmed. Resistance involves active countermeasures, while avoidance prevents exposure to the stress. Partly due to their sessile nature, plants have developed sophisticated metabolic responses to cope and survive, rather than avoiding stressful conditions as mobile organisms can. Stress can be defined by its negative effects on growth and development of the individual and can be external or internal. Internal stresses, such as spontaneous gene mutations or aberrant cell division might cause adverse effects on metabolic or genetic regulation. External stresses on plants can be divided into those of biotic or abiotic nature. Biotic stresses include pathogen attack, herbivory and competition. Abiotic stress arises from unfavourable environmental conditions, such as suboptimal temperature, water and nutrient availability, or light conditions. Stressful conditions can be a permanent state for the plant or they can be acute. Plants have adapted to permanent stress by altering their morphological features, such as succulence of their leaves, placement of their stomata, and specialization of tissues. To cope with acute stress plants have evolved responses that recognize the condition and subsequently set counteractive metabolic pathways in motion, such as in systemic acquired resistance against biotic stress or in the activation of heat shock proteins. Considerable knowledge has been gained over the last decade on physiological stress responses in plants involving individual proteins and genes. Much less, however, is known about the effect of stress on whole genomes.

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In the lecture delivered during the acceptance of her Nobel Prize, McClintock predicted large-scale genomic changes in response to unusual challenges including transposon activation and 'other structural modifications of the chromosomes' (McClintock, 1984). McClintock elaborated on four distinct examples of stress that could cause widespread genomic restructuring facilitated by transcriptional transposon activation, transposition of mobile elements and chromosome breakage-fusion-bridges. These four examples of stress include (1) tissue culture, (2) plant pathogen attack, (3) interspecies crosses, and (4) germline separation from somatic tissues during early development. While direct molecular evidence at the time was lacking we are now beginning to observe and understand stress-induced whole genome responses at the molecular level.

Here we review the evidence that has accumulated since McClintock's prediction that stress can cause the restructuring of the genome and revisit three of her examples: tissue culture, pathogen attack and interspecies crosses. In light of current research we also discuss examples of abiotic stress causing genomic responses, and offer new models for mechanisms explaining whole genome responses to the trauma of interspecies crosses.

WHOLE GENOME STRUCTURE: DYNAMIC OR STATIC?

Since McClintock's predictions that stress can cause whole genome changes genetic research has made tremendous advances. Before examining the effects that stress can have on the restructuring of the genome it is important to discuss what these structural changes might encompass. There is no clear definition of what constitutes the structural alterations to chromosomes predicted by McClintock. One could argue that large-scale recombination, chromosomal

breakage-fusion-bridge cycles, and loss of chromosomes or chromosome fragments result in major genomic and likely phenotypic changes. Such changes might be induced or facilitated by transposition of DNA elements. Additionally, changes in heterochromatin can have large effects on genome function. Heterochromatin was first cytologically defined as repetitive DNA that stained differently and appeared more condensed when viewed under the microscope. This condensed packaging is achieved by certain chemical modifications of the histones, around which the DNA is coiled, as well as modifications of the DNA itself. In most cases, such modifications in the heterochromatin are stably inherited and coincide with decreased transcriptional activity. Modifications that alter the DNA's activity without altering its basic nucleotide structure are referred to as epigenetic changes and include the chemical modification of DNA or histones, most commonly with methyl or acetyl groups.

Resetting the chromatin landscape through epigenetic changes may therefore cause large-scale genomic effects, and is tightly correlated with the transcriptional activity of genes, transposons and possibly non-coding RNAs. Before taking a look at genomic changes in response to stress we will discuss the normal contribution of epigenetic mechanisms to genome integrity and functionality.

Genome methylation and control of heterochromatin

The silencing of DNA sequences that are potentially hazardous to the organism is considered an important housekeeping function. Heterochromatin is commonly regarded as such 'silent' DNA. It consists of large regions of repetitive nucleotide sequences and transposons, many of which are more or less degenerate. At least some heterochromatic sequences serve an important role. While not coding for proteins, ribosomal RNA genes are needed for ribosome synthesis, while centromeres and telomeres are essential for the stability of chromosomes. Transcriptionally silent and densely packaged DNA is necessary for proper function during chromosome segregation and cell division. Transposons, however, must be suppressed because they constitute two dangers for the genome: (1) their repeated units can cause spurious homologous recombination; and (2) their ability to transpose can lead to disruption or misregulation of important genes. Both of these dangers are suppressed by heterochromatinization. Since the heterochromatic state is mitotically stable it serves as an epigenetic mark that designates these regions as heterochromatin through multiple cell cycles.

Heterochromatin in plants and mammals is densely methylated. The degree of DNA methylation often correlates with transcriptional silence. In plants, animals and fungi, DNA methylation is associated with cytosine residues that are followed by guanine (CG methylation). In plants, cytosine methylation is also common at cytosines in CNG sequences and can be found at any cytosine residue (asymmetric methylation).

First indications of a role between loss of DNA methylation and transposon activation were reported in maize (Chandler *et al*., 1986). Methylation changes in the promoter within the maize *Spm* element lead to changes in transposon activity, supporting the notion that demethylation could cause transcriptional activation and subsequent transposition of an affected element (reviewed in Fedoroff *et al*., 1995).

The importance of keeping a proper state of DNA methylation has most dramatically been shown by studying organisms mutant in their methylase activity. Mutations in the Dnmt1 methyltransferase of mice lead to the inability to maintain DNA methylation and are lethal. Plants mutant in the Dnmt1 homologue *MET1* display a widespread loss of DNA methylation and a severe pleiotropic phenotype (Finnegan and Dennis, 1993; Finnegan *et al*., 1996). In contrast to mice, homozygous *met1* plants are viable for several generations (Kankel *et al*., 2003). Plants also methylate their genome at CNG sites using a methyltransferase unique to plants called CHROMOMETHYLASE3 (CMT3). Mutations in *CMT3* result in loss of CNG methylation at centromeric repeats and at many transposons (Bartee *et al*., 2001; Lindroth *et al*., 2001; Tompa *et al*., 2002) with minor changes in CG methylation. Kato *et al*. (2003) examined the roles of MET1 and CMT3 in subduing transposon activity by sampling the frequency of transposition of a DNA transposon. This element displayed little transpositional activity in both single mutants, *met1* and *cmt3*. However, in the *met1;cmt3* double mutant high frequencies of transposition were observed. It seems possible, therefore, that both DNA methylases MET1 and CMT3 are partially redundant in their function to suppress transposition via CG and CNG DNA methylation, respectively. A third class of methylases, DOMAINS REARRANGED METHYLTRANSFERASES (DRM), shows similarity to the mammalian *de novo* methylase DNMT3 and is responsible for *de novo* methylation at asymmetric sites (Cao *et al*., 2000; Cao and Jacobsen, 2002). Besides controlling asymmetric methylation, DRMs are partially redundant with CMT3 in methylating CNG sites (Cao *et al*., 2003).

In addition to DNA methylation, the tails of histones H3 and H4 can be modified by methylation. Methylation is one of several histone modifications, including phosphorylation, acetylation and ubiquitination, whose role is the object of increasing interest. Histone methyaltion is best described for lysine residues K4 and K9, but is not limited to them, and transcriptional activity coincides with methylation at K4 and demethylation of K9 residues in the histone H3 (Jenuwein and Allis, 2001).

Interestingly, methylation of DNA and histones appear to be in close dependence on each other. Methylation of H3K9 is dependent on a histone methyltransferase known as SU(VAR)3–9 in yeast and KRYPTONITE (KYP) in plants (Jackson *et al*., 2002). DNA methylation at CNG sites is partially dependent on KYP, suggesting that histone methylation occurs prior to DNA methylation. Mutations in the *KYP* gene were further coincident with loss of methylation on retrotransposons *Ta2* and *Ta3* and transcriptional activation of these elements (Johnson *et al*., 2002). Methylated H3K9 allows the binding of heterochromatin protein 1 (HP1) (Lachner *et al*., 2001), a protein necessary for the maintenance of H3K9 methylation (Aargaard *et al*., 1999)

and is thought to help propagate heterochromatin along the chromosome (Grewal and Moazed, 2003).

Methylation of DNA and histones, however, is not solely dependent on the activity of methyltransferases. Vongs *et al*. (1993) isolated a mutant that showed decreased DNA methylation (*ddm1*) and phenotypic instability. This gene encodes a putative chromatin remodelling factor homologous to the *SWI2/SNF2* DNA helicase that is essential for maintaining proper DNA methylation and is probably part of chromatin-remodelling complexes. Activation of a Mu-like transposable element (MULE) was observed in *ddm1* mutants (Singer *et al*., 2001). To elucidate the molecular basis for *ddm1*-induced phenotypic instability, Miura *et al*. (2001) investigated a dwarf phenotype that arose in the *ddm1* background. They determined that the dwarf phenotype was caused by a transposon inserted into the *DWF4* gene, which encodes an enzyme required for brassinosteroid synthesis and normal cell elongation. Lack of DNA methylation had facilitated transposon insertion into the *DWF4* locus, thus causing an unstable dwarf phenotype with normal-looking sectors. The *ddm1* mutation also affects methylation of histone H3 (Gendrel *et al*., 2002). Loss of both DNA and histone H3K4 methylation is correlated with the transcriptional activation of several normally silent genes and transposons in the heterochromatic knob region of chromosome 4 in *Arabidopsis thaliana* suggesting that both DNA and histone methylation play a role in silencing regions containing DNA repeats.

In summary, methylation of both DNA and histone tails appears to be intimately involved in the maintenance or formation of heterochromatin. A change in the methylation landscape either via loss of DNA or histone methylation or a rearrangment of methyl groups within the genome can cause changes in gene transcription. And further, the loss of methylation is also correlated with transcriptional and transpositional activation of transposons, which in turn can cause gene mutations and phenotypic changes. Methyltransferases and chromatin remodelling factors appear to help in the addition and maintenance of methyl groups. In the next section we will look at the evidence for enzymes involved in actively demethylating the genome and thus causing an observed loss of methylation.

Demethylation of DNA

During DNA replication, DNA polymerase incorporates unmethylated cytosine opposite to guanine, leading to an overall reduction in methylation of DNA. The semiconservative nature of DNA replication results in maintenance DNA methylases recognizing hemimethylated symmetric sites (CG and CNG) and restoring full methylation. DNA methylation at asymmetric sites must be reapplied *de novo* after each replication cycle to the daughter chromatid that did not inherit the methylated C nucleotide. Failure of maintenance or *de novo* methylation leads to passive genome demethylation, as demonstrated in *met1* mutants.

Are there mechanisms that actively demethylate DNA? Several laboratories reported demethylation activity in cell extracts (Bhattacharya *et al*., 1999; Ramchandani *et al*., 1999), but clear evidence for a demethylase is still lacking. Glycosylases thought to function in DNA repair have been reported to remove m^5C thymine from DNA (Zhu *et al*., 2000). These enzymes excise mismatched or modified bases out of the sugar-phosphate backbone and replace them with complementary, unmodified nucleotides. The effectiveness of these glycosylases to act as efficient global demethylases has been questioned (Bird, 2002) but new evidence that glycosylases act as demethylating agents at specific sites in the genome has brought the issue back in discussion. Choi *et al*. (2002) reported the requirement of the DNA glycosylase *DEMETER* for reversal of endosperm imprinting in *Arabidopsis*. *DEMETER* works by activating the maternal copies of the *MEDEA* allele by demethylation of its promoter. This results in two active maternal and one imprinted paternal *MEDEA* gene, preventing overproliferation of the endosperm. *DEMETER* also regulates the gametophyte–specific expression of the normally imprinted (silenced) flowering time gene *FWA* in *Arabidopsis* (Kinoshita *et al*., 2004) and hypomethylation of repeat units in the promoter region has been linked to ectopic expression of *FWA* (Soppe *et al*., 2000).

There have also been reports that the paternal DNA in mouse embryos is subject to active demethylation soon after fertilization (Mayer *et al*., 2000; Oswald *et al*., 2000). While the authors did not identify a demethylation mechanism the authors reported demethylation to take place before the first round of DNA replication in the zygote, which is inconsistent with a passive loss of methylation due to a lack of maintenance methylation. The latter appears to be the case in frog embryogenesis where all gene transcription is stopped until after some 5000 cells have been formed, thus preventing production of methyltransferases, such as Dnmt, and methylation of the newly synthesized DNA (Bird, 2002).

Gene silencing

In the previous two sections we have examined the role that methylation plays in the heterochromatinization and transcriptional control of genes. Further, we have discussed evidence that methylation correlates with gene silencing. Following we will review and discuss historic and recent evidence that lead to the development of the current RNAi model of gene silencing.

Gene silencing was first noticed in petunia plants expressing a chimeric gene in which a strong constitutive promoter was fused to the chalcone synthase cDNA (Napoli *et al*., 1990). Surprisingly, the introduction of the transgene not only resulted in silencing of the transgene itself but also silenced identical endogenous sequences (Napoli *et al*., 1990). A connection between gene silencing and virus resistance was made in 1993 when tobacco plants transgenic for the tobacco etch virus (TEV) coat protein and infected with TEV were shown to recover from the infection (Lindbo *et al*., 1993). Previously infected plants even developed new healthy tissue, which was immune to re-infection with TEV but not to infection with other closely related viruses. It was further noticed that expression of the transgenic TEV coat protein gene was suppressed and

TABLE 1. *Proteins involved in silencing mechanisms*

Protein name	Arabidopsis homologue(s)	Function	References
DICER	DICER-LIKE (DCL) 1, 2, 3, 4	Cuts dsRNA into small fragments. microRNAs.	Schauer *et al*. (2002)
RNA-dependent RNA polymerase	RDR 1 to 6; also known as SDE1/SGS2	Amplifies microRNAs	Vaistij *et al*. (2002) Mourrain *et al*. (2000) Dalmay *et al*. (2000)
Histone H3 K9 methyl-transferase	SDG33 a.k.a. KRYPTONITE	Histone methylation of Lys 9	Shen (2001) Jackson *et al*. (2002)
Heterochromatin protein 1	CRD1, LIKE-HP1, a.k.a. TERMINAL FLOWER 2	Interacts with methylated histones	Bannister *et al*. (2001); Lachner *et al*. (2001)
Methyl binding domain protein	MBD1 to 13	Some MBDs recognize methyl-cytosine and can be associated with histone deacetylase	Zemach and Grafi (2003)
Methyltransferase	Methyltransferase (MET1)	DNA methylation at CG sites	Finnegan and Dennis (1993)
Chromomethylase	Chromomethylase (CMT3)	DNA methylation at CNG sites	Lindroth *et al*. (2001)
de novo methylase	Domains rearranged methylase (DRM)	*De novo* DNA methylase	Cao *et al*. (2000)
SWI2/SWI2 DNA helicase	Decreased in DNA methylation (DDM1)	Chromatin remodelling factor	Vongs *et al*. (1993)

that infection with the virus itself was not necessary to induce the virus-resistant state in the transgenic plants (Lindbo *et al*., 1993). This process, referred to as post-transcriptional gene silencing (PTGS), is functionally remarkably similar to the more recently described RNA interference (RNAi) pathway (Lindbo *et al*., 2001), which will be discussed in detail below.

As early as 1994 evidence had been reported that viroid RNA replication was sufficient to direct methylation to homologous sequences in the genome of tobacco (Wassenegger *et al*., 1994). This indicated that likely there was crosstalk between abnormal RNAs and the genome. In 1998, Fire and colleagues showed that injection of double-stranded RNA (dsRNA) interfered with expression of the endogenous homologous gene in *Caenorhabditis elegans* (Fire *et al*., 1998). Single-stranded sense or antisense RNA, when injected separately, however, only reduced transcript accumulation slightly. Subsequent studies involving mutants of several different species revealed an intricate pathway that most likely plays a role in three distinct processes in organisms as diverse as plants, fungi and animals: First, it is involved in normal development of the organism by aiding in post-transcriptional regulation (Kidner and Martienssen, 2003); secondly, it helps the host in defense responses against invading nucleic acids, such as those from viruses and transgenes (Napoli *et al*., 1990; Lindbo *et al*., 1993); thirdly, it represses transposons by initiating and maintaing condensed heterochromatin (Volpe *et al*., 2002; Schramke and Allshire, 2003).

Our understanding of the RNAi pathway is emerging from studies in diverse model systems ranging from plants, to *C. elegans*, to fungi and fruit files (for reviews, see Tijsterman *et al*., 2002; Grewal and Moazed, 2003; Table 1). According to the present model, double-stranded RNA (dsRNA) is produced in a variety of ways (see below) and serves as substrate for the enzyme Dicer, which cuts dsRNA molecules into approx. 22–26-nucleotide-long fragments. An RNA-dependent RNA polymerase amplifies these small RNAs, which are called small interfering RNAs (siRNAs) when targeted against invading viruses or transgenes and microRNAs (miRNAs) when targeted against endogenous genes. Subsequently, an RNAi silencing complex (RISC) or, in the case of DNA targets, RITS complex (from RNA-induced initiation of transcriptional gene silencing) (Verdel *et al*., 2004), that includes members of the *ARGONAUTE* (*AGO*) gene family, mediates the annealing of the small RNAs to the cognate RNA or DNA target (see Fig. 1). If DNA is the target, silencing may initiate through the recruitment of a histone methyltransferase that methylates lysine residue 9 on the amino-terminal tail of the nucleosome component histone 3 (H3K9). A specialized domain of histone methyltransferase, the chromo-domain, may bind small RNAs directly and thus target loci with complementary DNA. Methylated H3K9 (H3mK9) may subsequently recruit silencing factors, such as HP1 (SWI6 in *Schizosaccharomyces pombe*). In *S. pombe*, when SWI6 is bound to H3mK9, additional silencing factors and chromatin remodelers are recruited to silence heterochromatin (Grewal and Moazed, 2003). In plants, the activity of the cytosine methyltransferase CMT3 can further strengthen imprinting by adding methyl groups to the DNA. Spreading of heterochromatin is achieved by continuous DNA methylation starting at a 'nucleation centre' and perpetuating itself until it reaches so called 'boundary elements', which stop the spread of DNA methylation (Grewal and Moazed, 2003).

RNAi may do more than silence invasive nucleic acids. Recent studies suggest that the RNAi machinery is necessary for the maintenance of heterochromatin and the silencing of DNA repeat units (Volpe *et al*., 2002; Schramke and Allshire, 2003; Pal-Bhadra *et al*., 2004; Verdel *et al*., 2004), transposons, retrotransposons, and related 'evolutionary debris'. This maintenance of heterochromatin is thought to proceed via constitutive low-level transcription of sense and antisense RNAs (Volpe *et al*., 2002; Schramke and Allshire, 2003). The resulting dsRNA triggers the RNAi pathway and causes silencing of homologous loci. This mechanism of heterochromatic maintenance immediately

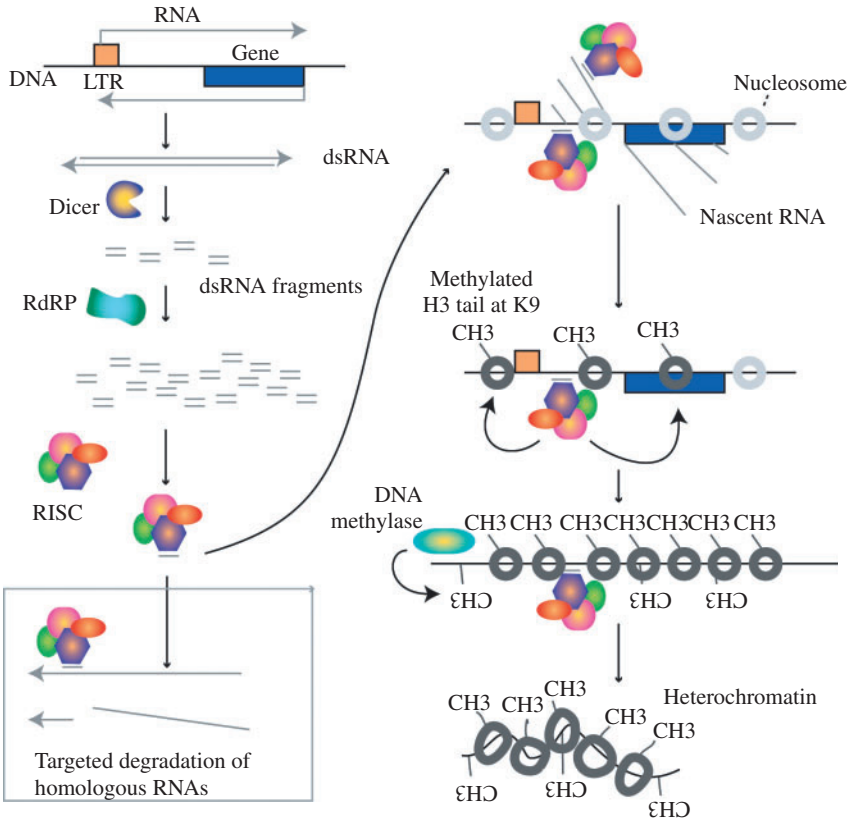

FIG. 1. Left: generalized RNAi pathway. Double-stranded RNA (dsRNA) produced, for example, by convergent transcription, serves as a substrate for the enzyme Dicer, which cuts dsRNA molecules into short (approx. 21–22 nucleotide long) fragments. An RNA-dependent RNA polymerase (RdRP) amplifies these small RNAs. The RNAi silencing complex (RISC) contains the ARGONAUTE (*AGO*) protein, mediates the annealing of the small RNA strands to the cognate mRNA and induces degradation or blocks translation. Right: action of RNAi on DNA. A specialized RISC complex (RITS) (Verdel *et al*., 2004) targets loci homologous to small RNAs for epigenetic suppression, presumably through recognition of DNA sequence or of nascent RNA, leading to recruitment of histone methylases, which add methyl groups to lysine residues (in particular in positions K9 and K27) on histone 3 (H3). Methylated H3K9 may recruit heterochromatin protein 1 or DNA methylases, which transfer methyl groups to the DNA and ultimately lead to heterochromatin formation. CH3 (inverted): methylated DNA; CH3 (right way up): methylated histones.

suggests a way to silence any kind of DNA repeats, especially those that might produce hairpin RNA or that are organized in opposite head-to-head arrangements or inverted repeats (see Fig. 2). In fact, RNAi failure in mutants causes the activation of transposable elements (Miura *et al*., 2001, 2004; Singer *et al*., 2001; Lippman *et al*., 2003) and will be discussed in detail later.

ELICITORS OF GENOMIC STRESS

In the previous part of this review we have discussed recent reports describing mechanisms that allow an organism to regulate global activity of large portions of its genome via epigenetic mechanisms such as methylation of DNA and histones. In this section we will review classic and recent evidence that support the notion that 'stress' can exert its effects on the organism not only via physiological response pathways but also via genomic, and indeed epigenetic, responses. We illustrate similarities in genomic stress responses of plants to four very different causes of stress: tissue culture, pathogen attack, abiotic stress and interspecific hybridization.

Tissue culture

During tissue culture single cells or tissue explants de-differentiate from the developmental state in the explanted tissue to form callus and then re-differentiate into new tissue types. This reprogramming of the genome 'inflicts on the cell a series of traumatic experiences' (McClintock, 1984). Tissue culture-induced genomic changes, as predicted by McClintock (1984), have indeed been found to be associated with several mutagenic mechanisms (Meins, 1983; Scowcroft *et al*., 1986; Lee and Phillips, 1988; Hirochika *et al*., 1993; Kaeppler *et al*., 2000). Hirochika (1993) was the first to report tissue culture-induced mobilization of the tobacco retrotransposons *Tto1*, *Tto2* and *Tnt1*. Later, Hirochika *et al*. (1996) reported the activation of a retrotransposon in rice after tissue culturing and Kikuchi *et al*. (2003) noticed activation of a miniature inverted repeat transposable element (MITE) after anther culture of rice. Activation of this MITE led to its transposition into new genomic locations. Tissue culture stress is associated with wounding during explant isolation and with cell wall hydrolases commonly used to isolate protoplasts by digesting cell walls prior to culturing.

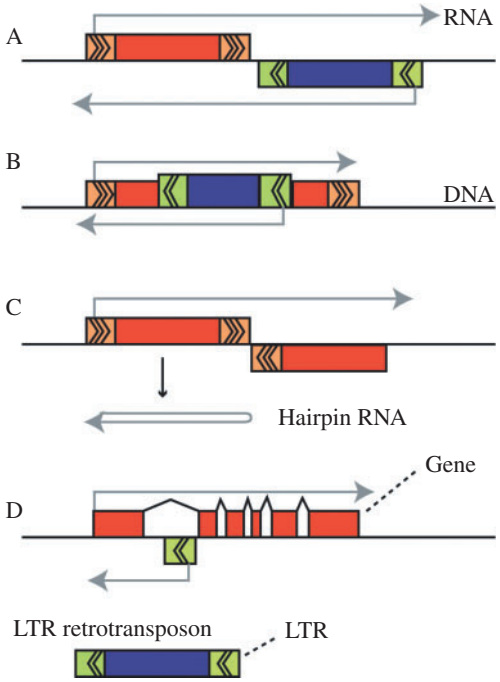

FIG. 2. Arrangements of long terminal repeat (LTR) retrotransposons and possible effects. LTRs can function as promoters or enhancers and drive expression not only of the associated retrotransposon genes, but also of adjacent genes. Transcription, indicated by the grey arrows, leads to synthesis of complementary RNAs, which anneal forming double-stranded RNA (dsRNA) and trigger RNAi initiation. (A) Two LTR retrotransposons are positioned in a head-to-head configuration and read-through RNAs of both elements result in dsRNA. (B) An LTR retrotransposon is inserted inside another causing the formation of partially overlapping complementary transcripts. (C) Inverted repeat formed by an LTR retrotransposon leading to an RNA with internal complementarity (hairpin). (D) A solo-LTR inside the intron of a gene. Solo LTRs are remnants of retrotransposons that have 'lost' the rest of their element. Similar scenarios have been observed in wheat (Kashkush *et al*., 2003).

Wounding might contribute to transposon activation (Grandbastien, 1998), but activation can also occur in the absence of enzyme treatment (Kubis *et al*., 2003) perhaps as a result from exposure to hormones in the culture medium. Hormones can indeed activate promoters of certain transposons (Takeda *et al*., 1999). A direct mechanistic link between tissue culture and transpositional activation, however, is still missing, although a decrease in DNA methylation has been observed (Kaeppler and Phillips, 1993; Kubis *et al*., 2003). In conclusion, it is likely that tissue culture compromises the epigenetic homeostasis of plant genomes and can result in secondary genomic effects.

Pathogen attack

Traditional plant pathogens. Plants have developed several genetically regulated lines of defense against pathogens. Plants use physical barriers, such as waxy cuticules, chemical antimicrobial substances, molecular surveillance by resistance genes, and cellular responses such as systemic acquired resistance (SAR) (for a review, see Dangl and Jones, 2001). Recently, RNAi has been identified as a major line of defense against viruses. Here we focus on RNAi and other epigenetic consequences of plant pathogen attack.

Pathogen attack is obviously a stressful event for plants and we know relatively little about its effects on genome integrity. The best evidence for pathogen-induced genomic shock comes from studies on barley stripe mosaic virus (BSMV), a tripartite, positive sense RNA virus that infects several grass species. It was noted that plants infected by BSMV displayed unusual instability such as frequent mutations. Molecular analysis of these mutations revealed that viral infection had mobilized transposons (Dellaporta *et al*., 1984; Mottinger *et al*., 1984; Johns *et al*., 1985). The mechanism of transposon induction by BSMV is unknown, but analysis of other viruses suggests candidates. It has long been noted that changes in phenotype are often associated with virus infection. A histone K27 SET-domain methylase has been found in a chlorella virus that infects *Paramecium* (Manzur *et al*., 2003). Although the presence of chromatin modifying enzymes had not been reported in plant viruses, several viruses encode proteins that suppress RNAi (reviewed in Baulcombe, 2002). The structure of one such suppressor resembles a molecular caliper that selectively binds and sequesters microRNAs of 20–22 base pairs (bp) in length (Vargason *et al*., 2003; Ye *et al*., 2003). The production of proteins that suppress RNAi by viruses clearly indicates that the plant RNAi response includes viruses as targets. In addition, it provides a possible explanation for the induction of epigenetic changes on the host during viral infection.

Infection by other pathogens, such as fungi and bacteria, is also stressful to plants and results in genomic responses. Infection by the fungus *Peronospora parasitica* increased genomic recombination three-fold (Lucht *et al*., 2002). This response is probably not specific to *Peronospora* since two different viruses induced a similar response in tobacco (Kovalchuk *et al*., 2003). It is possible that pathogen infection with its concomitant oxidative burst (Wojtaszek, 1997) may damage DNA triggering DNA repair. The extent of this response and its effects on epigenetic and genomic remodelling are unknown. Pathogen stress can activate retrotransposons (Grandbastien, 1998; Beguiristain *et al*., 2001). Treatment with fungal elicitors that hydrolyse the plant cell activate transcription of the tobacco Tnt1 retrotransposons (Grandbastien *et al*., 1997; Melayah *et al*., 2001). Takeda *et al*. (1999) reported an increase in activity of the retrotransposon Tto1 in response to wounding, treatment with methyl-jasmonate, fungal extracts and tissue culture. Tto5 expression was increased after treatment with salicylic acid or viral infection and Bs1 movement was also reported in maize after viral infection (for review, see Grandbastien, 1998).

Bacterial pathogens manipulate their host cells for the pathogen's advantage by secreting proteins that interact with host cell components (Greenberg and Vinatzer, 2003). Although studies to date have identified proteins that interact with the plasma membrane, chloroplasts or mitochondria of host cells, it is possible that some of the secreted proteins may target the genome or epigenetic

regulation. Indeed, candidates with nuclear targets are emerging (Szurek *et al*., 2001).

Bacterial pathogens in the genus *Agrobacterium* provide the best example of host genome modification. The transformation of host cells via insertion of the bacterial T-DNA, a process most likely derived from bacterial conjugation, represents one of the best described cases of horizontal gene transfer and it has been studied extensively (for review, see Escobar and Dandekar, 2003). Insertion of the T-DNA into the host genome is not always a precise and isolated event: major chromosomal rearrangements are often found in transformed genomes (Nacry *et al*., 1998; Laufs *et al*., 1999; Tax and Vernon, 2001; Forsbach *et al*., 2003) suggesting that T-DNA insertion either requires or causes genomic instability. *Agrobacterium* is not the only pathogen that transfers genomic sequences to the host: many plant virus genomes are found integrated in host plants, although some of these events may be rare and accidental (Harper *et al*., 2002).

In conclusion, pathogens can induce genomic remodelling and regulatory changes. The role of these responses is largely mysterious. Given the impact of host–pathogen interactions on the evolutionary path of plants, one can speculate that genomic effects may be the result of the war between the two organisms, as suggested by the finding of RNAi suppressors in viral pathogens.

Transposons. Together, DNA and RNA transposons comprise a large component of most genomes, and can be considered as intracellular pathogens. Indeed, the difference between transposons and viruses is blurred in the gypsy class of retroelements (Bucheton, 1995) and in other plant viruses and retrotransposons (Richert-Poggeler and Shepherd, 1997; Harper *et al*., 2002). RNA- or retrotransposons, also known as class I transposons, replicate via an RNA intermediate. This is in contrast to class II transposons (DNA transposons), which replicate via a cut-and-paste mechanism. Retrotransposons are divided into two subgroups. While all retrotransposons encode a reverse transcriptase some are flanked by long terminal repeats (LTRs) while others are not (non-LTR elements). Depending on the position of the gene for the enzyme integrase involved in transposition, the LTR-containing group can further be subdivided into Ty3/gypsy-like, or Ty1/copia-like retrotransposons (Galun, 2003). In contrast to terminal inverted repeats (TIRs) of class II transposons, LTRs are direct repeats, usually several hundred base pairs in length (Galun, 2003). LTRs contain the elements' promoter and enhancer (Pauls *et al*., 1994). Recombination can result in the separation of one LTR from the protein-coding sequences of the transposon it is flanking and produce so called Solo LTRs. Ty1/copia-like transposons initiate transcription within the LTR (Voytas and Boeke, 2002). This has important implications for the role that Solo LTRs may play in the genome with respect to the potential control of neighbouring genes. Transposons may also play an important role in the evolution of gene function and may be involved in the restructuring of genomes due to their ability to restructure or rearrange chromosomes (Agrawal *et al*., 2001; Witte *et al*., 2001).

There is ample evidence that transposons are kept in an inactive, silent state in the genome by heterochromatization (discussed above). This process often correlates with methylation of the element or its promoter region causing suppressive chromatin accumulation and subsequent silencing of the gene. Silent transposons are prone to mutations because no evolutionary pressure is exerted on them. Over time, genetic recombination or transposition can cause LTR transposons to assume head-to-head positioning. Figure 2 illustrates possible arrangements of LTRs or TIRs that if transcribed can lead to the formation of hairpin or dsRNA and potentially serve as a trigger for RNAi. LTRs can function as promoters or enhancers and drive expression not only of the retrotransposon element they flank, but also of adjacent genes (Michaud *et al*., 1994; Whitelaw and Martin, 2001; Kashkush *et al*., 2003). Schramke and Allshire (2003) provided evidence that interspersed LTRs can play a role in regulation of gene expression during development via RNAi. Studying *S. pombe*, these authors showed that a gene close to LTRs was silenced by RNAi-dependent chromatin silencing. In some cases, retroelement LTRs may be required to maintain the repression of genes involved in vegetative growth but not during meiosis. In this experiment the authors noticed that genes involved in sexual differentiation were often found adjacent to an LTR. All of these genes were normally repressed unless the organism was stressed by nitrogen starvation. In poor medium, nitrogen starvation is a signal for mating and cell division responses. When the authors tested the transcriptional activity of these normally stress-induced genes in single gene mutants of the RNAi pathway they observed strong de-repression under non-stress, high-nitrogen conditions. Schramke and Allshire (2003) concluded that a breakdown of RNAi caused the release of transcriptional repression of genes adjacent to LTRs. However, a direct link between nitrogen starvation and a temporary shut-down of the RNAi machinery in wild-type cells under nitrogen starvation has not yet been shown. From the evidence presented it seems possible that LTRs can drive low level transcription of sense RNA of transposons or adjacent gene transcripts which could induce gene specific RNAi. At the same time stress- or developementally induced transcription from normally inactive LTRs might turn on a different set of transcripts that could be in either sense or antisense direction of adjacent genes and lead to silencing or activation of genes, respectively. There is some evidence that retrotransposons are directly or indirectly regulated by developmental cues. Some elements are tissue specific in their transcriptional activity, such as Tnt1, which is normally transcribed in root tissues only and Tto1, Tos10 and Tos17 are not expressed in leaf tissue (Hirochika, 1993; Hirochika *et al*., 1996; Grandbastien, 1998). The PREM-2 element from maize appears to be preferentially transcribed in early developing microspores, and other elements (Opie, Huck, Cinful, BARE-1) are mainly expressed in leaf tissue (for review, see Grandbastien, 1998).

If genes adjacent to LTRs are induced indirectly via transposon activation the question remains how LTRs are induced in the first place. Stress-induced gene transcription is usually achieved via signal transduction pathways, which

can turn on defined stress responses, such as heat shock or the bacterial 'SOS' response. These responses require signalling ligands and signal transduction cascades ending with transcription factors that can fine-tune the response. Evidence presented above that implicates LTR-driven stress-induced transcription of transposon or adjacent gene sequences has generally come up short on detailed promoter analyses correlated with stress inducibility. Some exceptions, however, are studies on the promoter structure of Tnt-1, Tto1, BARE-1 and Tos17 (Suoniemi *et al*., 1996; Grandbastien, 1998). In addition, the snapdragon Tam3 promoter has been analysed in detail (Hashida *et al*., 2003). Takeda *et al*. (1998) showed that a 13-bp repeat element in the Tnt-1 LTR is required for transcriptional activation via treatment with methyl-jasmonate, fungal elicitors or for responsiveness to wounding and tissue culture. Homology analysis of the Tnt-1 promotor showed similarity to the promoter sequences of Tto1 and to that of a gene from asparagus involved in defense responses. Induction of stress-induced transposons could also be a secondary effect. As discussed above, transposons, as well as other genes, are effectively silenced by promoter methylation and (re-)-activated by demethylation (Fedoroff *et al*., 1995). It is therefore possible that stress causes demethylation and, indirectly, gene activation. Whether or not methylation changes in these cases are random or directed is uncertain. However, with the discovery of microRNAs and transcript-directed RNAi it is possible to envision ways how methylation might be directed to specific sequences leading to silencing of genes in the vicinity of these sequences.

In summary, stress has been reported as a factor in changing the transcriptional activity of LTRs and the genes they affect. Activation of transcripts adjacent to interspersed LTRs under stress conditions has been reported in mammals and fission yeast (Michaud *et al*., 1994; Whitelaw and Martin, 2001; Schramke and Allshire, 2003). Kashkush *et al*. (2003) reported LTR-dependent transcriptional activation of genes adjacent to LTRs in newly synthesized allotetraploid wheat. Although these examples at this time are sparse, they illustrate how transposons and multi-copy LTR elements might have genome-wide effects even if their activation is restricted to transcriptional activity without transpositional activity.

Abiotic stress

Like biotic stress, abiotic stress can lead to a host of genetically programmed responses resulting, if successful, in stress avoidance or stress tolerance. Here we focus on genomic, rather than physiological responses to stressful physical conditions.

In a few cases transposon activation in response to abiotic stress has been reported. The best studied example is the transpositional activity of *Tam* elements (transposable element of *Antirrhinum majus*), a temperature-sensitive class II transposon from snapdragon. Coen *et al*. (1986) characterized three snapdragon elements, *Tam1*, *Tam2* and *Tam3*. All of these elements were first isolated in flower colour mutants. Three mutant alleles of the *Nivea* gene, encoding a chalcone synthase, were isolated (Coen *et al*., 1986; Almeida *et al*., 1989) and one mutant allele of the *Pallida* gene, encoding an enzyme involved in the cyanidine pathway, resulting in red flowers. Each of these mutant alleles contained *Tam* element insertions in either their promoter sequences (*Tam1* and *Tam 3*) or in their coding sequence (*Tam2*). These transposon-induced mutations are unstable and lead to flower colour variegation. Interestingly, it was shown that the rate of excision was greatly dependent on lower than normal temperatures (15 °C) resulting in 1000-fold higher transposition than in higher temperatures (25 °C). Of the three *Tam* elements only *Tam3* shows this kind of temperature sensitivity but until today the molecular mechanism underlying this phenomenon has remained unclear. Kitamura *et al*. (2001) showed that low-temperature-induced *Tam3* activation was also dependent on the position of the *Tam3* copy in the genome, while silencing of transposition at higher temperatures appeared to occur simultaneously in all sampled loci. The position effect might be a function of binding affinity of the transposase to the TIRs of the element (Hashida *et al*., 2003). Hashida *et al*. (2003) sampled the methylation state of snapdragon DNA extracted from plants grown at 15 °C or 25 °C and found higher temperatures to result in hypermethlyation of DNA and lower temperatures to result in demethylation. Remarkably, the methylation state was reversible within one generation. Yamashita *et al*. (1999) analysed the 500-bp region surrounding the *Tam3* elements and noticed several hairpin structures, which might be targeted by methylation. Hashida *et al*. (2003) suggested that the temperature sensitivity of transposition activity of *Tam3* might be correlated with the methylation state via a temperature-sensitive DNA methyltransferase or that other proteins, whose expression is temperature-sensitive, associate with regions of the element recognized by a methyltransferase and block access to the DNA.

Jiang *et al*. (2003) reported the characterization of the first active MITE from rice. This element is a *Tourist-like* MITE and was named *mPing* (for miniature *Ping*). A second element, *Pong*, was also found to be active in rice cell cultures. Since active transposition appeared to be preferentially occurring in rice cultivars that had been adapted from their original tropical and subtropical locations for cultivation in cool climates, the authors speculated that this might be another example of temperature-induced genomic shock that might have helped the diversification of rice cultivars. Interestingly, one *mPing* transposed into a rice homologue of the flowering time gene *CONSTANS* (Jiang *et al*., 2003), exemplifying the effect transposition of stress-activated elements can have on the adaptation of a shocked genome to a change in the environmental conditions the organism is exposed to.

In *Medicago sativa* cold-induced transcriptional activation of multiple copies of a retrotransposon was observed. Interestingly, the cold-induced response was not concomitant with DNA demethylation (Ivashuta *et al*., 2002). Microclimatic changes were also implicated in retrotranposon activity of the barley *BARE1* retrotransposon (Kalendar *et al*., 2000). Kimura *et al*. (1999) reported transcriptional activation of a SINE RNA from silk worm to heat shock, cycloheximide treatment and viral infection.

A mysterious but fascinating case of abiotic stress-induced genome remodelling has been observed in flax. When certain varieties are exposed to varying environmental conditions they produce progeny that has different, but stably inherited characteristics (Cullis, 1973). These derivative strains, called genotrophs, appear to have numerous changes in DNA structure (Oh and Cullis, 2003).

To summarize, abiotic stress can result not only in well-programmed physiological stress responses but also in genome-wide changes. Stress-induced genomic responses include transposon activation, transposition, and structural genome changes. Like other stress responses transposon-mediated alterations in transcriptional activity of affected genes might lead to avoidance or tolerance of the stress. Unlike many other stress responses, however, transpositional activation appears to be a reaction not directly targeting an evolutionarily developed physiological pathway but is a hit-or-miss approach to finding an appropriate way of handling an unusual challenge.

Allopolyploidization and hybridization as a cause for genomic stress

The fourth example of a stress inflicted upon a plant is that of invasion of a foreign genome by way of fertilization with pollen of a different species. In most cases, plants have evolved barriers preventing the fusion of its gametes with those of individuals of a different species. However, interspecific hybridization between close relatives can occur, although it normally results in sterile offspring. But in some cases such as crosses in which chromosome doubling occurs before or immediately after hybridization, fertile progeny can arise, which, owing to the presence of duplicated parental genomes, are called allopolyploids. In allopolyploids intergenomic recombination of the homologous chromosomes (those contributed from the two different parental species) is infrequent (Comai *et al*., 2003). The chromosomes of the two original species are instead retained independently throughout subsequent generations. Allopolyploidization is an important process through which new species may theoretically arise quickly. However, estimates on how many species have arisen through allopolyploidy vary (for a recent review on the evolution of plant polyploids, see Liu and Wendel, 2003). Although direct comparisons between allopolyploids and their progenitors have only been conducted for few species (Liu and Wendel, 2003), established allopolyploids are often vigorous in growth and high in seed yield and fertility. Indeed, many of today's crop plants are of allopolyploid origin.

Synthetic allopolyploids of the model plant *Arabidopsis*, of wheat, and of cotton have been the focus of studies aiming to understand the molecular basis for the wide range of phenotypic variability associated with allopolyploidization (Feldman *et al*., 1997; Comai *et al*., 2000; Schranz and Osborn, 2000; Adams *et al*., 2003; He *et al*., 2003). Comai *et al*. (2000) reported phenotypic instability in early generations of allopolyploids of *Arabidopsis*, which were associated with widespread gene silencing and occasional gene activation. These instable phenotypes that varied between siblings included widely variable flower morphology, stem fasciation, variation in rosette leaves, and anthocyanin variegation of the stem (Comai *et al*., 2000). Gene silencing, often uniparental, was also found in the corresponding natural allopolyploid (Lee and Chen, 2001). In cotton, uniparental gene silencing was observed to switch during development and affected both recent and established allopolyploids (Adams *et al*., 2003). Gene silencing and, more rarely, activation have also been observed in wheat (Kashkush *et al*., 2002; He *et al*., 2003). Allopolyploidization was correlated with widespread changes in DNA methylation and with hypermethylation of silenced genes (Liu *et al*., 1998; Lee and Chen, 2001; Madlung *et al*., 2002), suggesting a causal relationship between the remodelling of the methylation landscape and stochastic alterations in gene activity and phenotype. In rapidly dividing embryonic or meristematic cells chromatin regulatory pathways might undergo differing paths of normalcy or malfunction, depending on fluctuations of critical factors or on the assembly of functional or misfunctional complexes. This can give rise to sectors containing tissue with cells of different epigenetic states. Such cells could be inherited through mitotic events potentially producing different phenotypes in siblings with identical genetic make-up, thus explaining the phenotypic variability observed in early generations of synthetic *Arabidopsis* allopolyploids (Comai *et al*., 2000; Madlung *et al*., 2002).

While transcriptional changes in synthetic allopolyploids have been reported in multiple systems, reports on genome structure changes have been largely confined to wheat. Since sequence rearrangements were reported by Song *et al*. (1995) in synthetic allopolyploids of *Brassica*, several studies have found evidence for widespread sequence elimination in first generations of newly synthesized allopolyploids of wheat (Feldman *et al*., 1997; Liu *et al*., 1998; Ozkan *et al*., 2001; Shaked *et al*. 2001; Kashkush *et al*., 2002). However, an extensive genomic survey of allopolyploid cotton has revealed no DNA changes (Liu *et al*., 2001). Some evidence points to allopolyploidization and hybridization as a cause for transposon activation. Comai *et al*. (2000) reported the transcriptional activation of a repeat unit in the 5′ region of RAP2.1, with similarity to transposons in newly formed allopolyploids of *A. thaliana* and *Arabidopsis arenosa*. Retrotransposon activation was also observed in rice hybrids (Liu and Wendel, 2000). Kashkush *et al*. (2003) observed transcriptional activation of retrotransposons in polyploid wheat. Interestingly, the expression patterns of some genes adjacent to transposon LTRs were also altered, lending support to the hypothesis that hybridization can alter the silencing state of heterochromatin and thus lead to activation of previously dormant genes. Direct evidence for hybridization-induced transposition via demethylation of elements, however, has so far not been reported.

In summary, stresses as diverse as those induced by tissue culture, biotic agents, abiotic conditions, or responses due to the stress of interspecific hybridization can result in whole genome changes. While biotic and abiotic stress responses often involve well-rehearsed metabolic pathways responses to genome hybridization are often genomic in nature. Consequences of interspecies crosses range from transcriptional

gene silencing, gene activation, methylation changes and transcriptional activation of transposons and coincide with phenotypic variability suggesting that epigenetic changes play a role in the novel phenotypes.

MECHANISMS ACTING DURING ALLOPOLYPLOIDIZATION

So far in this review we have described epigenetic housekeeping functions and their involvement in genome stability. We have then described four very different examples of stress and their effect on the whole genome structure of plants. The molecular mechanisms underlying the response of plant genomes to stress, however, are still unclear. In the last part of this paper we will attempt to (*a*) merge correlative evidence of stress and genome structure presented above and (*b*) review models for genomic responses to stress, focusing on allopolyploidization as one example of stress.

Allopolyploidy results from the hybridization of two different species. Doubling of the chromosomes either before hybridization (as in the combination of pollen and eggs from already autopolyploid parents) or immediately after zygote formation allows proper meiotic pairing, which often is impossible in diploid interspecific hybrids. Several reviews have discussed possible mechanisms leading to novel variation in polyploids (Comai, 2000; Osborn *et al*., 2003; Comai *et al*., 2003; Liu and Wendel, 2003; Riddle and Birchler, 2003). These models have examined the problems that might arise from the reunion of diverged parental genomes. One type of problem concerns the maintenance of separate parental genomes in the allopolyploid nucleus, which relies on enforcement of homologous recombination (Comai, 2000). Successful allopolyploids establish a strict separation of recombination between the two parental genomes (Comai *et al*., 2003). Relaxation of high homology criteria in recombination can lead to exchanges between homologous chromosomes and repeated sequences in the genome, potentially leading to loss of homology-dependent heterozygosity, and also forming aberrant chimeric chromosomes, deletions and duplications (e.g. dicentric).

Other types of problems concern regulatory interactions. Subunits of certain protein complexes may have diverged and become mismatched (Fig. 3) resulting in impaired function of the hybrid complexes (Comai, 2000; Osborn *et al*., 2003). The combination of diverged regulatory systems may produce unexpected expression patterns (Fig. 3), either because biparental contributions produce expression-altering dosages of factors, or because the interaction of regulatory factors from one parent and the target DNA sequence of the other are suboptimal (Osborn *et al*., 2003; Riddle and Birchler, 2003). Consistent, with these models a MYB-type transcription factor was found to contribute to species isolation in flies (Barbash *et al*., 2003) and enhancer traps showed frequent abnormal expression in fly hybrids (Hammerle and Ferrus, 2003). Last, the maintenance of a proper epigenetic environment is crucial for function and survival and allopolyploidization is assumed to bring about widespread alterations (Comai, 2000; Osborn *et al*., 2003; Comai *et al*. 2003; Liu and Wendel, 2003). It remains to be determined, however, how allopolyploidization causes epigenetic remodelling.

We previously discussed the emerging importance of RNAi in maintaining suppression of heterochromatic sequences. It is conceivable that the combination of diverged genomes might cause malfunctions in the RNAi pathways. A scenario for malfunction is suggested by uniparental expression of certain genes. Seitz *et al*. (2003) observed that, in mice, certain microRNAs were transcribed only in the maternal genome while a homologous retroelement was transcribed only in the paternal genome. They speculated that microRNA could regulate the retroelement, perhaps in parental imprinting or in selected tissues. Elaborating on this observation, microRNAs that are specific to the egg or the sperm may target heterochromatic elements within each allopolyploid parental genome to shut down undesired transcripts. It is conceivable that interspecific crosses could prevent silencing of species-specific transposons and cause altered gene expression in the offspring (see Fig. 3). Thus, micro-RNAs could play a role in parent-of-origin effects through maternal cytoplasmic contributions and effects potentially explained by parental dosage and perhaps by imprinting mechanisms have indeed been observed in crosses of *A. thaliana* with *A. arenosa* (Comai *et al*., 2000; Bushell *et al*., 2003; C. Josefsson and L. Comai, unpubl. res.). RNAi requires the regulated expression of ARGONAUTE, DICER, an RNA-directed RNA polymerase, the RISC complex proteins and chromatin remodelling factors (see Fig. 1). This regulation presumably requires the concerted action of several transcription factors. The RNAi pathway could malfunction due to dosage unbalance of regulatory factors, and defective subunit interaction (Comai *et al*., 2003; Osborn *et al*., 2003, Riddle and Birchler, 2003).

De-methylation of the genome causes transcriptional activation of genes and transposon activation (Miura *et al*., 2001, 2004; Singer *et al*., 2001; Lippman *et al*., 2003). There is some evidence that methylation changes occur in response to genomic stress inflicted upon newly formed allopolyploids of *Arabidopsis* (Lee and Chen, 2001; Madlung *et al*., 2002) or in interspecific and intergeneric hybrids of rice (Liu and Wendel, 2000; Xiong *et al*., 1999) and wheat (Shaked *et al*., 2001).

McClintock referred to transposons as 'controlling elements' (McClintock, 1968) predicting that their presence and activity was controlling genes in their vicinity. Direct evidence for this role has been limited, yet tantalizing. Alteration in transposon activity was shown to affect neighbouring genes of *Spm* (Masson *et al*., 1987) and *Mu* insertions (Martienssen *et al*., 1990) in maize. Evidence also exists for a role of LTRs in affecting the regulation of adjacent genes (Whitelaw and Martin, 2001; Kashkush *et al*., 2003). Activation of transposons (transcriptional and transpositional) has been shown in mutants, which are deficient in their ability to maintain proper DNA methylation (Singer *et al*., 2000; Miura *et al*., 2001; Gendrel *et al*., 2002). How can activation of genes that are normally silent in both parental genomes be explained? And how can silencing of normally active genes be explained?

A model is suggested by the interspersion of transposons such as LTR among genes. Suppose that genes A and A′ are

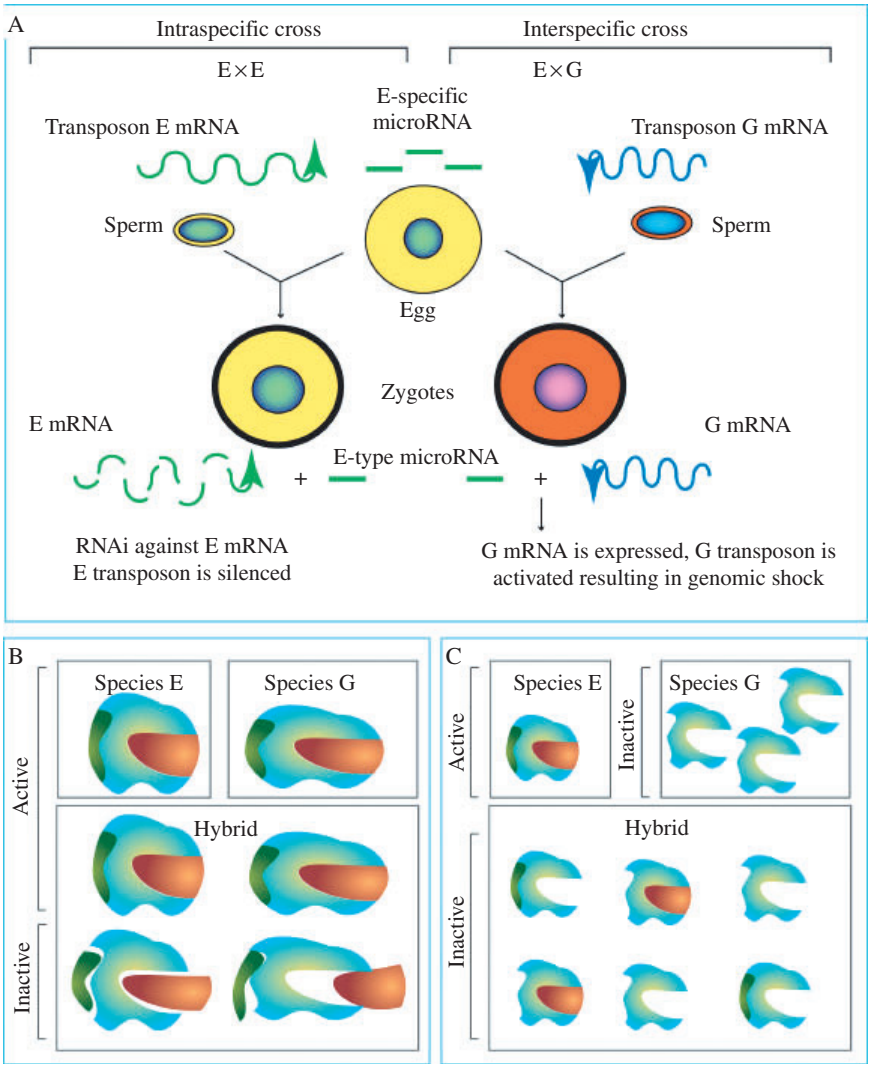

FIG. 3. Three hypothetical examples of parental incompatibilities. (A) The cytoplasm of the egg-parent (species E) contributes a microRNA specific for a paternally expressed transposon. The intraspecific cross leads to RNAi. In the cross of the egg-parent (species E) to a different species (G) the lack of a microRNA specific to a transposon expressed in the G parent prevents transposon suppression, triggering genomic instability. (B) A multi-protein complex involved in heterochromatic regulation has diverged since the separation of two species from a common ancestor. In intraspecific crosses all protein subunits are compatible. In interspecific crosses productive and unproductive assemblies take place depending on the random combination of protein subunits. If these complexes act at very low concentration inside the cell, their activity would vary stochastically in meristematic cells, in some reaching a critical threshold for activity and producing different imprinted states. (C) Genes encoding the subunits of the same complex illustrated in B have diverged in expression between species A and species G. Species G expresses higher amounts of the blue subunit and lower amounts of the green and brown subunits. In the hybrid, the excess of blue subunit titrates the substoichiometric amounts of green and brown subunits inactivating the complex.

homologous genes of related species. A is transcribed while A′ is silent. In addition, a Solo LTR 3′ of A′ could produce an antisense transcript of A′, but normally does not because of suppressive heterochromatin. After the union of the two genomes in a newly formed allopolyploid, the Solo LTR becomes activated forming antisense A′ RNA. The concurrent formation of sense RNA from A and antisense RNA from A′, produces dsRNA and RNAi could lead to silencing of A in the offspring.

Activation of heterochromatic transposons is consistent with the often stochastic nature of phenotypic instability and gene silencing in newly formed allopolyploids. Nevertheless, convincing evidence for this hypothesis is still lacking. Where should it be found? A consideration is that recent allopolyploids that are one or a few generations old, may represent a selected minority of zygotes where genome upheaval has been avoided, contained, or where it has yielded favourable changes. It is possible that the many failing zygotes formed upon hybridization experience far more drastic epigenetic change but are not easily sampled experimentally.

CONCLUDING REMARKS

We are beginning to understand the importance for epigenetic regulation in stress responses. Evidence to date

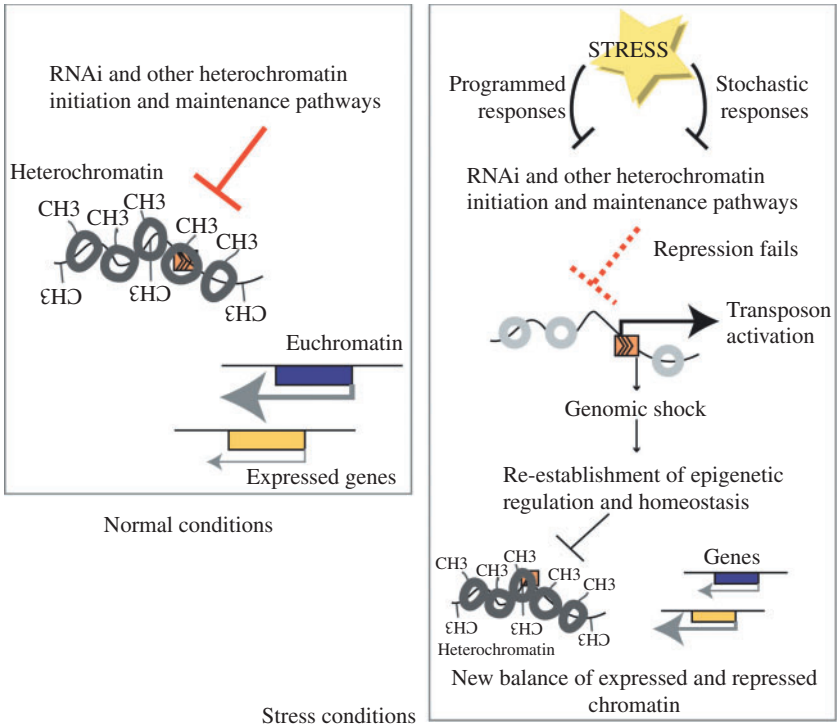

FIG. 4. Hypothetical genome regulation under normal and stress conditions. Under normal conditions heterochromatin maintenance mechanisms repress transcription of repetitive DNA (left). Stress can cause the relaxation of epigenetic imprints. RNAi and other heterochromatin maintenance pathways fail, resulting in the activation of transposons (right). If the shock does not lead to death the cell undergoes a stochastic remodelling of its genome resulting in altered epigenetic marks and in novel gene expression. CH3 (inverted): methylated DNA; CH3 (right way up): methylated histones.

suggests the following hypothetical model for stress-induced genomic shock (Fig. 4): epigenetic pathways are both targets of programmed responses and of stochastic malfunction caused by stress. Relaxation of epigenetic imprints affects the genome to a degree proportional to the degree of impairment and to the number of pathways affected. The result is improper expression of sequences that should normally be silenced. Expression of these sequences leads in turn to a silencing response as the cell attempts to regain regulatory balance. If the cell survives the shock and its immediate outcome, its genome will have undergone epigenetic remodelling, and often a genetic remodelling caused by DNA rearrangements and transposition. Through the action of selection, only epigenetic and genetic arrangements that promote fitness are maintained in the population.

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