Nuclear Stress Bodies

Giuseppe Biamonti¹ and Claire Vourc'h²

¹Istituto di Genetica Molecolare. CNR; Via Abbiategrosso 207. 27100 Pavia, Italy

²Université Joseph Fourier; INSERM; Institut Albert Bonniot U823, La Tronche BP170, 38042 Grenoble cedex 9, France

Correspondence: biamonti@igm.cnr.it and claire.vourch@ujf-grenoble.fr

Nuclear stress bodies (nSBs) are unique subnuclear organelles which form in response to heat shock. They are initiated through a direct interaction between heat shock transcription factor 1 (HSF1) and pericentric tandem repeats of satellite III sequences and correspond to active transcription sites for noncoding satellite III transcripts. Given their unusual features, nSBs are distinct from other known transcription sites. In stressed cells, they are thought to participate in rapid, transient, and global reprogramming of gene expression through different types of mechanisms including chromatin remodeling and trapping of transcription and splicing factors. The analysis of these atypical and intriguing structures uncovers new facets of the relationship between nuclear organization and nuclear function.

Nuclear stress bodies (or nSBs) were discovered in the late 1980s and very soon after were associated with cellular response to stress agents (Mahl et al. 1989, Sarge et al. 1993). They are transient subnuclear organelles clearly distinct from other nuclear bodies (Cotto et al. 1997). High-resolution electron microscopy analysis has revealed the peculiar and complex organization of nSBs that appear as highly electron-dense structures frequently adjacent to chromatin blocks. The electron-dense core structure consists of a large number of perichromatin granules (PGs) and is surrounded by individual PGs that seem to enter or exit the central core (Chiodi et al. 2000). The function of nSBs is still largely unknown, however, it is commonly accepted that they correspond to highly packed forms of ribonucleoprotein

complexes. nSBs are rarely detectable in unstressed cells; their number drastically increases after heat shock as if specific processes involved in the production and/or maturation of specific RNAs were altered in stressed cells. Another distinguishing feature of nSBs is their specificity for human and primate cells (Denegri et al. 2002). Further molecular characterization proved that nSBs originate from the unexpected transcription of large pericentromeric heterochromatic blocks triggered by transcription factors involved in the cell response to stress (Jolly et al. 2004, Rizzi et al. 2004). Intriguingly, these RNAs remain close to the sites of transcription and probably exert their function by recruiting specific factors and affecting chromatin organization. Thus nSBs are at the convergence of several important aspects of

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cell biology such as the epigenetic control of gene expression, noncoding RNAs, and control of RNA splicing activities (reviewed in Biamonti 2004; Jolly and Lakhotia 2006; Eymerly et al. 2009a).

THE DAWN OF NUCLEAR STRESS BODIES: THE TWO FACES OF MOLECULAR JANUS STRUCTURES

Even a limited increase of the growth temperature of a few degrees Celsius, referred to as “heat shock,” induces in all cell types and organisms, a series of functional and morphological alterations, which are gradually reversed over a period of several hours once the physiological temperature is restored. Heat shock leads to an immediate and almost complete block of important cellular processes such as DNA replication and transcription. At the posttranscriptional level, heat shock transiently inhibits pre-mRNA splicing, nucleo-cytoplasmic transport and translation (Morimoto and Santoro 1998). The mechanisms underlying splicing inhibition are still poorly understood. In 1989, Mahl et al. proposed that heat shock could act by perturbing the integrity of ribonucleoprotein complexes, i.e., the substrates of splicing and of nuclear export. Upon thermal stress, a subset of hnRNP (heterogeneous ribonucleoprotein particles), which are the main protein constituents of ribonucleoprotein complexes, were seen to be recruited to specific nuclear sites which, as visualized by electron microscopy, appear to be enriched in highly packed forms of ribonucleoprotein complexes called “perichromatin granules” (PGs) (Mahl et al. 1989). This was the first report of what we now know as nuclear stress bodies or NSBs.

A few years later, another protein, heat shock factor 1 (HSF1), was shown to form a small number of nuclear granules after different types of stress conditions (Sarge et al. 1993). The cellular response to adverse environmental and physiological conditions such as heat shock, or an exposure to amino acid analogs, heavy metals, oxidative stress, anti-inflammatory drugs, or arachidonic acid, leads to a rapid and transient activation of genes encoding heat

shock proteins (hsps) and molecular chaperones (reviewed in Lindquist 1986 and in Christians et al. 2002). Stress-induced transcription is regulated by a family of heat shock transcription factors (HSF). In vertebrates, four members of the HSF gene family (HSFs 1-4) have been characterized (reviewed in Pirkkala et al. 2001), each mediating the response to distinct forms of cellular stress, including HSF1, which responds to the classical inducers of the heat shock response. Whereas in unstressed cells HSF1 is maintained unbound to DNA, after heat shock, it undergoes reversible oligomerization into a DNA binding competent trimer. Two distinct mechanisms, involving negative regulatory domains and phosphorylation, cooperate to control the activity of this factor (reviewed in Cotto and Morimoto 1999). In higher eukaryotes, HSF1 trimers appear within minutes of activation and bind to specific heat shock elements (HSE) in the promoters of heat shock genes (hsp genes). In 1993, Sarge et al. showed that full activation of HSF1, induced by heat shock, cadmium sulfate or by the amino acid analog L-azetidine-2-carboxylic acid, also results in the accumulation of this factor into a small number (four to six) of nuclear granules with a maximum diameter of 2–2.5 μm (Sarge et al. 1993) (Fig. 1). Intriguingly, these granules were described only in monkey and human cells and were not observed in rodent cells (reviewed in Jolly and Lakhotia 2006). HSF1 granules were also characterized independently (Cotto et al. 1997; Jolly et al. 1997) as novel entities, distinct from other subnuclear compartments. Their kinetics of formation and disappearance depends both on the nature and on the severity (duration, and concentration or intensity) of the stressing agent. More importantly, the number of bodies correlates with cell ploidy. On the basis of this latter finding it was suggested that HSF1 granules could be assembled on specific chromosomal targets and may represent stress-dependent transcription sites. However, the lack of colocalization with the classical hsp genes, such as hsp70 and hsp90, initially argued against this model (Jolly et al. 1997). A few years later the hypothesis was revitalized by the *in vivo* analysis of HSF1 granules with HSF1-GFP

Figure 1. nSBs visualized with anti HSF1 and anti acetylated lysine antibodies. In unstressed cells (NHS), HSF1 (green labeling) displays a diffuse nucleocytoplasmic distribution. Upon heat-shock (HS), HSF1 is redistributed to a few nuclear foci, also known as stress granules, which form primarily on the 9q12 loci (indicated with arrow heads). An average of three copies of chromosome 9 are present in HeLa cells. In heat-shocked cells, the 9q12 locus is enriched in acetylated histones. The binding of HSF1 is followed by the formation of acetylated foci, the recruitment of RNA polymerase II, the subsequent transcription of sat III repeats mainly into “G rich” transcripts, and the ultimate recruitment of splicing factors. (Bar scale = 5 μ m).

(Jolly et al. 1999a). This analysis showed that successive, short rounds of heat shock, induced cycles of assembly/disassembly of HSF1 granules. Interestingly, granules always formed in the same nuclear positions as if bound to an underlying immobile matrix. These experiments, therefore, raised the question about the nature of the chromosomal targets involved.

Soon after the first description of HSF1 granules, several studies by the group of Biamonti made the link between these structures and the nuclear bodies containing RNA binding proteins, which had previously been identified by Fuchs et al. (Mahl et al. 1989). These studies started with the characterization of a novel hnRNP protein called hnRNP A1 interacting protein—HAP, identified by others as Saf-B, scaffold attachment factor B (Renz and Fackel-mayer 1996), or as HET, the Hsp27-ERE-TATA-binding protein (Oesterreich et al. 1997). After a mild heat shock, HAP/Saf-B is recruited to a small number of nuclear bodies. Importantly, HAP bodies coincide with HSF1 granules, highlighting the double nature of these bodies containing both transcription factors and proteins involved in pre-mRNA metabolism (Weighardt et al. 1999). We now know that HSF1 granules

and HAP bodies define two different functional states, partially overlapping in time, of what we now call nuclear stress bodies or nSBs (reviewed in Biamonti 2004). Although HSF1 granules form at the onset of the heat shock response and rapidly disappear during the recovery period following heat shock, HAP bodies become visible after 1 hour of mild heat shock, with a maximum size reached after 3 hours of recovery. The formation of HAP bodies requires ongoing transcription (Weighardt et al. 1999) and these structures are sensitive to RNase treatment (Chiodi et al. 2000), suggesting that RNA is a major component of nSBs.

The importance of RNA in the assembly of nSBs was further supported by the ultrastructural analysis of nSBs (Chiodi et al. 2000), which appeared as clusters of perichromatin granules surrounded by compact chromatin (Charlier et al. 2009). PGs in the bodies are specifically labeled by antibodies against hnRNP HAP and contain nascent bromouridine-labeled RNA. These structures are clearly distinct from nuclear speckles, where a number of pre-mRNA processing factors also accumulate.

A subset of splicing factors of the SR family, including SF2/ASF, SRp30 and 9G8 are efficiently recruited to nSBs, whereas the distribution of other members of the same family, including SC35, the standard marker of nuclear splicing speckles, is not affected by stress (Jolly et al. 1999b; Denegri et al. 2001).

THE COMING OF AGE OF NUCLEAR STRESS BODIES

Several studies clarified the nature of nSBs, unveiling unexpected links with the epigenetic organization of large chromosomal blocks and with noncoding RNAs.

Nuclear Stress Bodies Assemble on Blocks of Satellite III DNA

In 2002 C. Jolly and C. Vourc’h directly addressed the nature of the chromosomal targets on which nSBs are assembled (Jolly et al. 2002). By investigating the distribution of HSF1 on metaphase chromosome spreads they found

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that, in all heat-shocked cells, HSF1 binds to the extended pericentric heterochromatic q11-q12 region of human chromosome 9. This region, also known as secondary constriction or 9qh region, is a large block of heterochromatin primarily composed of long tandem arrays of Sat III repeats (Jones et al. 1973). Although the Sat III consensus sequence does not contain canonical HSE elements, purified HSF1 binds to a genomic Sat III fragment (Grady et al. 1992) from human chromosome 9 but not to other satellite repetitive sequences (i.e., Sat II or α -satellite) in in vitro assays (Jolly et al. 2004), demonstrating the existence of direct interaction of HSF1 with Sat III sequences. Intriguingly, HSF1 binding does not require preliminary stress-induced chromatin reorganization because an HSF1 mutant, deleted in its carboxy-terminal *trans*-activation domain, constitutively binds to the 9qh region in unstressed cells (Jolly et al. 2004).

Meanwhile, the group of Biamonti exploited a completely different strategy to identify chromosomal regions involved in the assembly of nSBs (Denegri et al. 2002). Taking advantage of the fact that nSBs occur in human but not in rodent cells they used human-hamster somatic cell hybrids to identify human chromosomes that direct the assembly of nSBs in hamsters cells. In addition to chromosome 9, which represents the primary target of nSBs formation, they identified two other human chromosomes, 12 and 15, that are positive in this assay. With the same approach they narrowed the region of chromosome 9 required for the formation of nSBs to the 9q12 band identified by Jolly and Vourc'h. This chromosomal band, therefore, acts as the recruiting for nSBs.

Transcriptional Activation of Pericentromeric Heterochromatin

Altogether these findings paved the way for more work on this subject, which brought researchers to the crossroads between epigenetics and non-coding RNAs. Most of the data available at that time suggested that nSBs could be large transcription factories. However, this hypothesis was in conflict with the fact that the 9q12 region

was described as a region of noncoding constitutive, and therefore transcriptionally inactive, heterochromatin (Kokalj-Vokac et al. 1993). The idea of a transcription factory gained momentum, however, when it was shown that nSBs were enriched in acetylated histones (Fig. 1), an epigenetic mark of transcriptionally active chromatin, and did not contain typical heterochromatin markers such as HP1 proteins or histone H3 tri-methylated on lysine 9 (Rizzi et al. 2004, Jolly et al. 2004). Moreover, it was also observed that HSF1 binding, through the corecruitment of the histone acetyl transferase CREB binding protein (CBP), initiated a series of events involving chromatin remodeling, the recruitment of RNA pol II, but not of pol I or III, and culminated with the production of Sat III transcripts, which bound to several splicing factors (Jolly et al. 2004, Rizzi et al. 2004, Metz et al. 2004).

Nuclear Stress Bodies are Part of a General Response to Stress

The expression of Sat III sequences in heat-shocked cells now represents one of the best-documented examples of transcriptional activation of pericentric heterochromatin in metazoan cells. In addition to heat shock, the expression of Sat III sequences can also be induced by the amino acid analog azetidine and by a variety of physical (UV-light) and chemical (Cadmium sulfate) stressors known to activate HSF1 (Valgardsdottir et al. 2008, Sengupta et al. 2009), indicating that this event is part of the gene expression program controlled by HSF1. The kinetics and the extent of the induction vary with the nature of the stress agent, probably reflecting the robustness of HSF1 activation. Interestingly, in all cases Sat III RNAs remain associated with nSBs as they do in heat-shocked cells (Jolly et al. 2004) and are undetectable in the cytoplasm (Jolly et al. 2004; Valgardsdottir et al. 2005), as if these molecules exerted their action on chromatin.

HSF2, an isoform of HSF1 that forms heterotrimers with HSF1 and modulates its activity (Sandqvist et al. 2009), is also present in nSBs where it binds to DNA in an HSF1-dependent manner (Alastalo et al. 2003). Depletion of

HSF2 leads to an increase of the heat induced transcription of Sat III sequences whereas, intriguingly, elevated HSF2 expression, mimicking what is observed in development, activates Sat III transcription in unstressed cells (Sandqvist et al. 2009). Although the functional implication of the HSF2/HSF1 interaction is still largely undefined, this result clearly indicates that Sat III expression may play a role in various physiological settings. This idea is also suggested by the observation that another transcription factor, the tonicity enhancer binding protein (TonEBP), also directs the formation of nSBs and the transcription of Sat III sequences in response to hyper-osmotic stress (Valgardsdottir et al. 2008). Notably, TonEBP is physiologically crucial for the formation and function of kidney protecting cells in the renal inner medulla from extraordinarily high levels of NaCl and urea. Putative binding sites for this factor are present in the Sat III sequence. Thus, distinct signaling pathways elicited by different stress inducers and acting through different transcription factors lead to the production of Sat III RNAs (Valgardsdottir et al. 2008). This is suggestive of an active role of Sat III sequences in the cell response to stress.

Interestingly, besides environmental stress, other physiological and pathological conditions lead to the activation of pericentric sequences in human cells; however, in none of these cases has the presence of nSB-like structures been documented. Frequently, the expression is linked to a change in the epigenetic organization of Sat III sequences and pericentric regions in general. For instance, expression of Sat III sequences is observed in cells treated with 5-Azacytidin, a potent inhibitor of DNA methylation (Eymery et al. 2009b). The expression of pericentric transcripts also occurs during replicative senescence at late passages of both primary fibroblasts and cancer cells (Enukashvily et al. 2007; Eymery et al. 2009b). The fact that an alteration of the heterochromatin structure may favor an accumulation of Sat III transcripts is also suggested by observations made in fibroblasts from patients affected by the Hutchinson–Gilford progeria syndrome (HGPS), in which a complete loss of the heterochromatic marks is accompanied by the expression of

chromosome 9 specific Sat III sequences (Shumaker et al. 2006). Another example is expression of Sat III transcripts in human testis, suggesting that Sat III transcripts may be somehow involved in the differentiation of germinal cells (Jehan et al. 2007, Sandqvist et al. 2009). These findings, together with a recent study showing expression of Sat III transcripts in embryonic cells (Faulkner et al. 2009), potentially link the expression of Sat III sequences to developmental programs.

The occurrence of transcription in normally silent portions of the genome, regardless of the formation of specialized nuclear structures, raises a number of questions about the role of pericentric heterochromatin and the impact of their transcriptional awakening. Does activation of Sat III arrays participate in global stress-induced, genome-wide down regulation of genome expression through transient sequestration of transcription factors? Alternatively, does activation of Sat III facilitate transcription of nearby genes through *cis*-acting effects or by creating nuclear domains in which genes escaping heat-induced transcriptional repression would relocate? Is gene expression also affected by transient targeting of specific splicing factors to Sat III RNAs? Finally, why do noncoding Sat III RNAs remain associated with, or in proximity to, arrays of SatIII sequences in the human genome? As discussed later, an answer might be that transcripts of pericentric origin are known to play a role in the establishment and maintenance of heterochromatin structure. Addressing these questions will be a major subject of future investigation.

THE ELUSIVE NONCODING SATELLITE III RNAs

The function of the SatIII transcripts in nSBs is unknown but they do have several intriguing properties. First, heat shock drastically induces the strong expression of polyadenylated Sat III RNAs, mainly corresponding to the G-rich strand (the presence of GGAAT repeats in Sat III transcripts imposes a difference in the G/C content between the two complementary DNA strands) (Jolly et al. 2004; Valgardsdottir et al. 2005 and 2008). It is worth noticing that

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transcription of Sat III sequences occurs even in unstressed cells (Valgardsdottir et al. 2008). Because of the repetitive nature of the transcripts and uncertainties about their size (see later discussion), it is impossible to assess the level of Sat III RNA molecules, which, however, appears to be low. The expression of Sat III sequences in unstressed cells may change our view of the role of pericentric heterochromatin, and is consistent with the observation that a subset of Sat III DNA sequences exists in an open, transcriptionally permissive state of chromatin in human cells (Gilbert et al. 2004). Another important aspect is the fact that the kinetics of accumulation of Sat III transcripts depend on cell type and the nature of the stressing agent. After mild heat shock (1 hour at 42 °C) the level of these RNAs peaks at 2–3 hour of recovery. However, because of their stability, the level of Sat III transcripts are still higher than in unstressed cells after one day of recovery (Jolly et al. 2004 and Valgardsdottir et al. 2008). Unlike protein coding transcripts, Sat III transcripts always remain associated with—or in close proximity to—the locus from which they originate (Jolly et al. 2004).

Discrepancies exist between estimates of the length of the Sat III transcripts. According to some authors (Jolly et al. 2004), Sat III RNAs are larger than 10 Kb whereas others (Rizzi et al. 2004) have observed that Sat III RNAs have a broad size distribution with most of the molecules falling between 5 and 2 kb. The possibility that shorter transcripts could derive from long precursors through post-transcriptional processing, i.e., splicing, remains to be established. The level of the expression induction of these sequences has also recently been measured by real time RT-PCR (Valgardsdottir et al. 2008), showing an induction between 10,000- and 100,000-fold for G-rich and 50-fold for C-rich Sat III sequences, and a peak at 2 h of recovery. Because of the repetitive nature of Sat III sequences, the actual induction of Sat III RNA molecules is certainly lower than this value. Because of our lack of knowledge concerning the structure of the repetitive Sat III arrays, it is not yet clear whether the production

of Sat III RNAs is driven by a canonical promoter. It is also conceivable that transcripts start randomly in the proximity of HSF1 molecules bound to HSE elements. Finally, nothing is known so far about the fate of these transcripts and the machinery involved in their degradation.

AN OPEN CHALLENGE: FINDING (A) FUNCTION(S) FOR nSBs AND SAT III TRANSCRIPTS

Sat III sequences have appeared late in evolution (Jarmuz et al. 2007). They are specific to the Hominoidea superfamily and are present on most human chromosomes. Their recent evolution accounts for the fact that Sat III RNAs and nSBs, or similar recruiting centers for RNA processing factors, are not found in rodent cells. This raises questions about the function of these sequences and suggests that other noncoding RNAs may play similar functions in different species. Interestingly, structures comparable to nSBs were found in *Drosophila* cells and called ω -speckles (Prasanth et al. 2000). Similarly to Sat III RNA, ω -speckles control the dynamics of pre-mRNA processing factors in heat-shocked cells. This evolutionary convergence of otherwise different systems (ω -transcripts derive from a single copy gene rather than from repetitive satellite elements) indicates that nSBs could be involved in splicing regulation.

Molecular Traps for Transcription and Splicing Factors?

nSBs may be viewed as transcription factories comprising a natural amplification of RNA polII promoters. Although the number of transcription units is not yet defined, both the extent of the Sat III arrays and the size of HSF1 foci suggest that thousands of transcriptional units may be simultaneously activated. Thus, the massive concentration of factors involved in the activation of Sat III sequences such as RNA polII or the histone acetyl transferase CBP may result in the transient sequestration of transcription factors from the surrounding nucleoplasm. This is in agreement with the

observation that, in heat-shocked cells, the formation of nSBs is accompanied by a global deacetylation of chromatin in the rest of the nucleus (Fritah et al. 2009). A similar hypothesis can be proposed for factors involved in pre-mRNA processing. Sat III RNAs are stable components of nSBs and mediate the recruitment of a number of proteins involved in pre-mRNA maturation (Denegri et al. 2001; Metz et al. 2004). Some RNA binding proteins are recruited through protein-RNA interactions (Chiodi et al. 2004). The recruitment of the splicing factor SF2/ASF is mediated by the RRM2 (RNA recognition motif)-domain, which is critical for its activity in alternative splicing (Metz et al. 2004, Chiodi et al. 2004), whereas other RNA binding proteins, such as hnRNP HAP, are recruited through protein-protein interactions (Denegri et al. 2001). Notably, although not accumulating in nSBs, other essential components of the splicing machinery, i.e., the snRNPs, are associated with Sat III RNAs (Metz et al. 2004). This is in line with the hypothesis that Sat III RNAs undergo at least some steps in the splicing reaction. In this context it is worth recalling that the splicing reaction is either blocked or delayed by thermal stress (Yost and Lindquist 1986; Bond 1988; reviewed in Bond 2006). It is intriguing that nSBs correspond to large clusters of PGs. Although the nature of PGs has not yet been elucidated, it has been suggested that they could contain aberrant RNAs blocked at early stages of maturation and engaged in degradation (Cervera 1979; Puvion and Viron 1981). Disruption of RNA maturation at an early stage of the process, as occurring after heat shock or in the presence of transcription inhibitors, could block RNA on its DNA matrix, leading to the formation of PGs.

Although the heat shock response correlates with a global shut-down of transcription and with an alteration of splicing functions (Yost and Lindquist 1986; Bond 1988; reviewed in Bond 2006), it is not entirely clear whether it affects the majority of pre-mRNAs, whether all transcripts are affected to a similar degree, or whether heat shock targets only specific subsets

of pre-mRNAs. Whatever the extent of this phenomenon, the activation of Sat III sequences could contribute to the shutdown or reprogramming of gene expression.

Alternative splicing affects more than 90% of cellular transcripts. Splicing profiles are controlled by the relative abundance of antagonistic hnRNP and SR proteins. It is plausible that Sat III RNAs, by sequestering specific RNA binding proteins into nSBs, may shift splicing decisions, as for instance toward the synthesis of molecules involved in the cell defense to stress (Fig. 2A).

nSBs are not the only nuclear bodies whose assembly involves a specific RNA molecule. This is also the case of paraspeckles, which are sub-nuclear foci found adjacent to nuclear splicing speckles, that contribute to regulation of gene expression by trapping adenosine to inosine (A to I) hyperedited RNA within the nucleus (reviewed in Bond and Fox 2009). Similarly to nSBs, formation of paraspeckles involves the interaction of the NEAT1 (also known as MEN- ϵ/β or VINC-1) noncoding RNA (ncRNA) with several RNA binding proteins, i.e., members of the DBHS (*Drosophila melanogaster* behavior, human splicing) protein family, consisting of PSPC1, P54NRB/NONO, or PSF/SFPQ. As for nSBs, the RNA moiety (NEAT1 ncRNA) is essential for the maintenance of the body and is also the nucleating factor (Sasaki et al. 2009, Clemson et al. 2009, Chen and Carmichael 2009, Sunwood et al. 2009). Indeed, paraspeckles form in early G1 near to the NEAT1 gene locus and are often found clustered near the NEAT1 gene in interphase. There are also important differences between the two bodies. Contrary to heterogeneous Sat III RNAs, NEAT1 is a well-characterized transcript encoded by a single-copy gene locus. Moreover, nSBs remain always associated or in close proximity with the sites of transcription of Sat III RNAs whereas paraspeckles can leave NEAT1 transcription sites to associate with nuclear speckles. The mechanisms allowing the existence of paraspeckles at distance from the sites of NEAT1 transcription are still unknown.

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Figure 2. Schematic illustration of the possible roles of nSBs in heat-shocked cells. nSBs are thought to play a role in the cellular response to stress and cell protection. Three main hypotheses have been proposed which are not mutually exclusive: (A) Control of transcription and splicing activities. Upon heat-shock, sat III sequences and transcripts are thought to play a role in the control of transcriptional and splicing activities through sequestration of transcription and splicing factors (both represented as dots). Transient trapping of these factors could contribute to the shutdown or reprogramming of gene expression. It is also plausible that Sat III RNAs, by sequestering specific RNA-binding proteins into nSBs, may influence splicing decisions toward the synthesis of molecules involved in the cell defense to stress. (B) Regeneration of heterochromatin structure. In fission yeast, transcripts from pericentric regions play a role in the formation and maintenance of heterochromatin. In human cells, sat III transcripts may also play a role in protecting heterochromatic pericentric regions following heat-shock, either as long RNA molecules or as small RNA molecules generated by the RNAi machinery. (C) Transcriptional de-repression of genes located in the vicinity of nSBs through position effects. Loss of epigenetic repressive marks (red flags) at the 9q12 locus following heat shock could abolish the transcriptional repression exerted by pericentric heterochromatin on the activity of promoter genes present in *cis* (here visualized in brown and green) or possibly in *trans* (not shown) through chromatin opening and binding of transcription factors (TF).

A Role in Heterochromatin Assembly and/or Maintenance?

RNA appears to have a major role in the assembly of heterochromatin (Maison et al. 2002; Muchardt et al. 2002). Two mechanisms have been so far identified through which RNA may act. In *Schizosaccharomyces pombe*, small-sized pericentric transcripts, generated through the processing of longer RNAs by the endoribonuclease Dicer, target the RITS (RNA induced transcriptional silencing) complex to complementary DNA sequences and direct the assembly of heterochromatin (Verdel et al. 2004; Buhler et al. 2006). One can speculate that, similarly, Sat III transcripts may be processed into small dsRNA, which would then be recruited to the human RITS complex (reviewed in Eymery et al. 2009a). In support of this hypothesis, in a chicken–human cell hybrid containing human chromosome 21, a loss of Dicer leads to the accumulation of pericentric specific transcripts and results in cell death and premature sister chromatid separation (Fukagawa et al. 2004). Moreover, real time RT-PCR indicates that, both in unstressed and heat shock cells, the level of C-rich Sat III RNAs is drastically lower than that of the complementary G-rich molecules (Valgardsdottir et al. 2008). This difference in complementary transcripts clearly argues against the existence of long double stranded Sat III RNAs that, as in *S. pombe*, would then be processed by Dicer. Moreover, so far, no evidence of short Sat III RNAs in human cells has been found.

The establishment of heterochromatin can also involve long noncoding RNAs through still poorly understood mechanisms. This is the case for the Xist transcript involved in female X chromosome inactivation (reviewed in Heard 2004). Because Sat III transcripts are stable transcripts that remain associated with the 9q12 regions even through the G2/M transition (Jolly et al., 2004), one can speculate that long Sat III RNAs may be involved in a similar process. Finally, it has been suggested that Sat III RNAs may have a role in stabilizing chromosomal regions that are prone to instability and rearrangements (Bartlett et al. 1988; Lamszus et al.

1999; reviewed in Robertson and Wolffe 2000 and in Duker 2002; Reshmi-Skarja et al. 2003) (Fig. 2B).

Impact of nSBs on Nuclear Organization

The transcriptional activation of pericentric sequences could profoundly affect the functional organization of the cell nucleus. Pericentric chromatin is thought to influence the expression of genes, either on the same chromosome or within the nuclear volume, through *cis* or *trans* mechanisms. Several examples have been reported in the literature in which gene inactivation is indeed associated with repositioning of repressed genes in the vicinity of these large blocks of heterochromatin. Based on these observations a model has been proposed in which repositioning of repressive genes in the vicinity of heterochromatin would be necessary for the maintenance of their repressed status through a position effect mechanism (reviewed in Fisher et al. 2002; Francastel et al. 2001; Zhimulev and Belyaeva 2003). It is conceivable that transcriptional activation of Sat III sequences may impact on the activity of other genes associated either on the same chromosome or in the nuclear space. This could be one of the multiple ways by which stress may induce a transient reprogramming of gene expression profiles (Fig. 2C).

CONCLUDING REMARKS

Although nSBs have not revealed all of their nature and function, a picture emerges today of a large center for the recruitment of transcription and splicing factors, involved in the global control of gene expression. If this hypothesis proves to be correct, it would represent a new remarkable illustration of the ingenuity of the mechanisms implemented by the cell to quickly adapt to environmental changes, and ensure its survival. Our poor knowledge of the structure of Sat III encoding regions and related transcripts still represents a barrier to functional analysis. However, combination of *in situ* approaches to determine the kinetics of formation and disappearance of nSBs, coupled

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with epigenetic and transcriptomic genome-wide approaches can now be performed to determine the impact of nSBs formation on global changes of gene activity in the course of the stress response. Answering this question represents an important and exciting challenge for the next decade.

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