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Nuclear stress granules: the awakening of a sleeping beauty?

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Nuclear stress granules are subnuclear compartments that form in response to heat shock and other stress stimuli. Although many components of nuclear stress granules have been identified, including HSF1 and pre-mRNA processing factors, their function remains a mystery. A paper in this issue describes the stress-induced transcriptional activation of one of the nuclear stress granule target sites, a heterochromatic region that has been considered silent (Jolly et al., 2004). These intriguing findings will certainly give the research of these structures a new twist.

Nuclear stress granules were originally described as subnuclear structures into which HSF1, a member of the heat shock transcription factor family regulating heat shock genes (Pirkkala et al., 2001), concentrates upon heat shock (Sarge et al., 1993). The nuclear granules are considered as discrete nuclear structures because they do not colocalize with other subnuclear bodies, such as nucleoli, PML bodies, coiled bodies, SC35-containing speckles, kinetochores, nuclear domains formed by nuclear matrix proteins like lamins, or sites for DNA replication (Cotto et al., 1997; Jolly et al., 1997; Weighardt et al., 1999; Chiodi et al., 2000). Although the nuclear stress granules were discovered a decade ago and have been the focus of intense research, their structure and function have remained enigmatic. It has been suggested that they serve as sites of storage and/or recycling of transcription factors (Jolly et al., 1997), and specifically, that they could act as shuttling centers where the distribution of HSF1 molecules, representing different activation states, is regulated (Jolly et al., 1999). According to another hypothesis, the nuclear granules may protect hypersensitive sites of the genome from stress (Jolly et al., 1999, 2002). The current experimental evidence, however, does not adequately explain the above-proposed functions of nuclear stress granules. A tangible clue to stress granule function is presented in this issue (Jolly et al., 2004), and elsewhere (Rizzi et al., 2003), providing evidence that the nuclear stress granules correspond to active transcription sites and that HSF1 could be a key determinant of this transcriptional activity.

When visualized by indirect immunofluorescence in cells exposed to heat shock, a major portion of the HSF1 pool localizes to large and brightly staining granules (Sarge et al., 1993; Cotto et al., 1997). Other protein-damaging stresses, such as heavy metals, amino acid analogues, and proteasome inhibitors, also induce the formation of HSF1-containing granules. In size, the HSF1 granules vary between 0.3 and 3 μm and appear as either single globular granules or grape-like clusters of smaller globular structures (Jolly et al., 1999). A remarkable feature of the HSF1 granules is their dynamic formation. Using live cell imaging, HSF1 can be detected in granules within 30 s of heat shock (Jolly et al., 1999). During attenuation and recovery from stress, the HSF1 granules gradually disappear (Cotto et al., 1997; Holmberg et al., 2000). The rapidity of the HSF1 translocation in response to stress indicates an energy-dependent and strictly regulated process, encouraging researchers to further pursue the biological relevance of HSF1-containing stress granules.

Although the appearance of nuclear stress granules temporally coincides with the DNA-bound and transcriptionally active form of HSF1, granules do not colocalize with the transcription sites of the known heat shock genes (Jolly et al., 1997). Based on early studies, several features of the HSF1 granules indicated a genomic target, but identifying the sites of granule formation was a hard nut to crack. An important clue was provided by Jolly et al. (2002), who, by using immunofluorescence on metaphase spreads from human fibroblasts, were able to show that the HSF1 granules assemble on the q11-q12 region of chromosome 9. Specifically, HSF1 is targeted to a nucleosome-containing subclass of satellite III repeats via a direct protein–DNA interaction (Jolly et al., 2002). Simultaneously, Denegri et al. (2002) reported the same target region and also suggested that the centromeric regions of chromosomes 12 and 15 could be targets for the HSF1 granules. Whether there are still other unidentified sites of nuclear stress granule formation in the genome remains to be elucidated.

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Abbreviations used in this paper: HAP, hnRNP A1–associated protein; HSF1, heat shock factor 1.

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Although HSF1 was the first component found in the nuclear stress granules, it has been proven not to be the only one. Surprisingly, HSF2, an enigmatic heat shock factor that has previously been considered refractory to stress stimuli, was recently shown to colocalize together with HSF1 to the nuclear stress granules in response to heat shock (Alastalo et al., 2003). The laboratory of Giuseppe Biamonti has provided evidence that a novel hnRNP protein, hnRNP A1–associated protein (HAP), translocates to the nuclear stress granules upon heat shock (Weighardt et al., 1999). HAP is a multifunctional protein involved in RNA metabolism, transcription, and nuclear structure (Denegri et al., 2001). With respect to HSF1, HAP is recruited to the nuclear stress granules with delayed kinetics and remains associated with the granules for a longer time during recovery (Weighardt et al., 1999).

Other RNA processing factors that have been reported to localize to the nuclear stress granules include hnRNP M, Sam68 (Src-activated during mitosis), and a subset of SR (serine-arginine) splicing factors (Jolly et al., 1999; Weighardt et al., 1999; Denegri et al., 2001). The need for RNA synthesis and the presence of splicing factors in the nuclear stress granules have raised the possibility that these structures are involved in transcription and splicing events (Chiodi et al., 2000; Denegri et al., 2001). In particular, the presence of HAP and Sam68 led researchers to relate them to the Sam68 nuclear bodies (SNBs), into which Sam68 is concentrated under normal conditions (Denegri et al., 2001). The resequestration of splicing factors from SNBs to nuclear stress granules could reflect the need for stress-induced alternative processing of specific transcripts.

The paper by Jolly et al. in this issue presents a breakthrough in the research on the nuclear stress granules, and is of great interest to the transcription field as a whole, as it demonstrates transcriptional activity within a locus that has been regarded constitutively heterochromatic and therefore silent. The stress-induced transcription of the satellite III repeats within the 9q11-q12 locus, which corresponds to a site of nuclear stress granule formation, is HSF1 dependent. HSF1 is also required for the acetylation of all core histones (H2A, H2B, H3, and H4) and accumulation of RNA polymerase II in the granules. The transcripts, which are large and stable, remain associated with the 9q11-q12 region even during recovery from stress and throughout mitosis. Transcriptional activation of a heterochromatic region of the human genome is further supported by Rizzi et al. (2003), who report the stress-dependent accumulation of acetylated histone H4 and detect transcription of the satellite III repeats at the 9q11-q12 locus. However, the transcripts identified by Rizzi et al. are heterogenous in size, ranging from 2 to 5 kb, whereas the transcripts detected by Jolly et al. seem very large and unable to migrate into a 1% agarose gel. The kinetics of histone acetylation differs markedly between the two papers. Rizzi et al. report acetylation of histone H4 in the nuclear stress granules that peaks at 6 h of recovery from heat shock, while acetylation of histones described by Jolly et al. is down-regulated already at 3 h of recovery.

The function of the transcripts generated in the nuclear stress granules is unknown. The observation that treatment with RNA polymerase II inhibitors and RNase A abrogates HAP localization to the nuclear stress granules (Weighardt et al., 1999; Chiodi et al., 2000) indicates that the transcripts could play a role in targeting HAP, and possibly other RNA processing factors, to the nuclear stress granules. This could also explain the delayed recruitment kinetics of HAP. Alternatively, the transcripts could be involved in the negative regulation of gene expression, as recent data demonstrate that the organization of silent domains, such as pericentric heterochromatin (Maison et al., 2002; Muchardt et al., 2002) and the inactivated X chromosome (Avner and Heard, 2001; Cohen and Lee, 2002), rely on noncoding RNA molecules. In yeast, RNA molecules encoded by centromeric repeats have been shown to influence chromatin architecture, by participating in the direct formation and maintenance of heterochromatin through RNA interference (Volpe et al., 2002).

In writing, the function(s) of the nuclear stress granules still remains a case unsolved. Therefore, the above recapitulated results will undoubtedly stimulate research on how and why these structures are formed, which components they hold, and by which timing and hierarchy these components are recruited. Also unclear is how activated HSF1 is distributed between the sites of heat shock gene transcription and nuclear stress granules, and whether HSF1 indeed is the master regulator of the granules. Furthermore, the functional relationship between transcripts and pre-mRNA processing factors needs to be established. This set aside, the finding that the reported transcripts arise from the 9q11-q12 region, which is thought to be constitutively heterochromatic, is highly intriguing. This region is now given an epigenetic status typical of euchromatic regions (Jolly et al., 2004; Rizzi et al., 2003). Taken into account that heterochromatin, which has been regarded as transcriptionally silent, constitutes a large portion of the eukaryotic genome, the obvious question to be addressed is whether the observed transcriptional activity is an exception. If not, the nuclear stress granules could herald the emergence of an entirely new role for heterochromatin. In fact, we may be about to see the awakening of a sleeping beauty.

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