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C. elegans sirtuin SIR-2.4 and its mammalian homolog SIRT6 in stress response

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Stress is a significant life event. The immediate response to stress is critical for survival. In organisms ranging from the unicellular *Saccharomyces cerevisiae* to protozoa (*Trypanosoma brucei*) and metazoan (such as *Caenorhabditis elegans*, *Homo sapiens*) stress response leads to the formation of cytoplasmic RNA–protein complexes referred to as stress granules (SGs). SGs regulate cell survival during stress by the sequestration of the signaling molecules implicated in apoptosis. They are a transient place of messenger ribonucleoproteins (mRNPs) remodeling for storage, degradation, or reinitiation of translation during stress and recovery from stress. Recently, we have identified chromatin factor, the sirtuin *C. elegans* SIR-2.4 variant and its mammalian homolog SIRT6 as a regulator of SGs formation. SIRT6 is highly conserved NAD^+-dependent lysine deacetylase and ADP-ribosyltransferase impacting longevity, metabolism, and cancer. We observed that the cellular formation of SGs by SIRT6 or SIR-2.4 was linked with the cell viability or *C. elegans* survival and was dependent on SIRT6 enzymatic activity. Here, we discuss how SIR-2.4/SIRT6 influences SGs formation and stress response. We suggest possible mechanisms for such an unanticipated function of a chromatin regulatory factor SIRT6 in assembly of stress granules and cellular stress resistance.

Stress Granules and Their Speculated Function

Stress granules form naturally when a cell deals with a variety of environmental stresses, including heat shock, oxidative stress, hyperosmolarity, viral infection, and UV irradiation.[1] They are non-membranous cytoplasmic foci ranging in size from 0.1–2.0 μm, containing non-translating messenger ribonucleoproteins (mRNPs), ribosomal subunits, eukaryotic initiation factors (eIF) 2, 3, 4, poly mRNA, polyA binding proteins (PABP, PABPC1), the argonaute proteins, microRNAs, mRNA-editing enzymes, RasGAP-associated endoribonuclease (G3BP), and proteins implicated in neurodegenerative or neurologic diseases such as ataxin-2 (ATX-2), survival motor neuron protein (SMN), the fragile X mental retardation protein (FMRP).[1-6]

Stress granules are found in plants, yeast, protozoa, and metazoan, in both cultured cell lines and intact tissues.[2] In *C. elegans*, stress granules have been observed in the cytoplasm of somatic as well as germline cells in response to stress (e.g., heat shock).[7] Unlike in mammalian cells, in which SGs and processing bodies (P-bodies) are spatially separable, in *C. elegans* SGs interact with P-bodies and germ granules.[1,7-9]

P-bodies are distinct from germ granules, and represent a second class of RNA granules that behaves differently in somatic and germline cells. Analyses

in *C. elegans* have shown that certain P-body components co-localize with germ granules and with two markers of stress granules, poly (A) binding protein (PABP) and TIA-1 presented in the oocyte large ribonucleoprotein (RNP) foci.[9] These protein–protein interactions suggest that stress granules, P-bodies, and germ granules may function together to regulate the balance of translated, repressed, and degraded mRNAs in germ cells.[9]

The dynamic nature of stress granules suggests that they are sites of mRNA triage, wherein mRNAs are dynamically sorted for storage, degradation, or translation during stress and recovery.[2] SGs have also been shown to regulate the stability of selected mRNAs and control the survival of stressed cells through recruitment of signaling proteins, transcription factors, RNA helicases, nucleases, and kinases into SGs.[1,2]

In cultured cell lines, the assembly/disassembly of SGs shows age-related changes in response to stress.[10] In contrast, little is known about the mechanisms governing the assembly of SGs in response to environmental stimuli in the physiological intact animal, in tissue and organ systems, or in both germ cell and somatic cell lineages.

Sirtuin sir-2 Gene Family is Evolutionary Highly Conserved in Amino Acid Sequence, Function, and Interaction Partners

Intriguing factors linking cellular activities to stress- and age-related diseases are silent information regulator 2 (SIR-2) proteins, also termed sirtuins. Sirtuins have a function in diverse cellular pathways, regulating transcriptional repression, aging, metabolism, cell defenses against DNA damage, and apoptosis.[11,12] Sirtuins appear to be activated in the nucleus as well as in the cytoplasm as part of a beneficial cellular response to stress, resulting in cell survival and extended lifespan in yeast, worms, flies, and mice. However, more recent studies have called the results with *C. elegans* and *D. melanogaster* into question because mechanistic studies have not converged on a specific activity coupled to aging.[13]

Recently, we have shown that assembly and disassembly of stress granules components in mammals and P-granules components in *C. elegans* can be regulated by a chromatin factor, the *C. elegans* sirtuin SIR-2.4 variant and its mammalian homolog SIRT6. SIRT6 possesses an ADP-ribosyltransferase and NAD^+-dependent deacetylase activity of both acetyl groups and long-chain fatty-acyl groups.[14] SIRT6 plays an important function in cellular homeostasis by regulating DNA repair, telomere maintenance, glucose, and lipid metabolism, thus contributing to the overall longevity and health of the organism.[14-21] Role of sirtuins in stress granules assembly and survival has not been determined, although stress granules contain a large number of post-translationally modified proteins that control the assembly of the granules.[22]

Mammals possess seven sirtuins, SIRT1-SIRT7. The *C. elegans* genome encodes four sirtuins, SIR-2.1 through SIR-2.4, thus offering an excellent model system to analyze sirtuins stress-stimulated mechanisms of action in a multicellular organism.[23] Whereas the majority of studies in the worm have focused on the mammalian SIRT1 homolog, SIR-2.1, due to its role in lifespan determination and germline silencing, SIR-2.2, 2.3, and 2.4 are largely uncharacterized.[24,25] We have recently described the function, localization, and interactome of the mitochondrial SIR-2.2 and SIR-2.3 as well as of the nuclear SIR-2.4 (**Table 1**).[26,27] As shown (**Table 1**), the sirtuins are an evolutionarily conserved protein family regarding their amino acid sequences, localization, and interaction partners.

SGs Formation is an Evolutionarily Conserved Aspect of C. elegans SIR-2.4/Mammalian SIRT6

We and others observed that SIR-2.4 is required for normal stress resistance in the worm.[9,27] We found a correlation between the survival rate of *sir-2.4* mutant animals and the number of P-granules in germline cells implying a function of SIR-2.4 in the regulation of lifespan and P-granules formation in response to stress.[27] Under non-stress conditions, endogenous SIR-2.4 was primarily found in the nucleus, associated with the nuclear envelope and P-granules in germ cells.[27] Following heat shock, SIR-2.4 rapidly and reversibly redistributed into cytoplasmic P-granules structures. Interestingly, P-granules share many properties with mammalian stress granules and P-bodies. For example, the number of P-granules is increased in stressed and aged animals.[2,9,26,29] We have shown that the changes in size and number of P-granules were altered by growth temperature and by SIR-2.4. Furthermore, we observed that P-granules proteins (e.g., PGL-1/3 and GLH-1.2) specifically interact with SIR-2.4.[27] Additionally, we demonstrated that SIR-2.4 in somatic cells is also recruited to bright cytoplasmic foci (granules) in response to heat shock. In order to examine SIR-2.4 regulation in vivo, we performed yeast two hybrid screen (Y2HS). We found that *C. elegans* SIR-2.4 variant interacts with a protein encoded by cDNA clone K08F4.2. K08F4.2 encodes a protein that possesses a high amino acid sequence similarity to mammalian G3BP (the Ras-GTPase activating enzyme). Thus, our data fit quite well with our studies showing that mammalian SIRT6 protects cells against heat-shock through the interaction with G3BP.[27]

In mammalian cells, we observed that SIRT6 shifted from a predominant nuclear localization to both nuclear and cytoplasmic when stressed by heat shock or sodium arsenite treatment, and that SIRT6 indirectly regulates dephosphorylation of G3BPSer149, an important step during SG formation.[30] Similar to the situation in *C. elegans*, loss of SIRT6 affected stress granules formation and decreased mouse embryonic fibroblasts (MEFs) viability. In particular, the granules were reduced in the size after heat shock in SIRT6-deficient MEFs in contrast to wild-type MEFs. Furthermore, addition of the sirtuin inhibitor nicotinamide (NAM), which inhibits deacetylase and

mono-ADP-ribosyltransferase activities, also altered the size of stress granules in wild-type MEFs similar to those in cells lacking SIRT6, thus implying important function of SIRT6 enzymatic activity in the assembly and disassembly of SGs. Interestingly, the SIRT6 functions on stress granules assembly were not found with other sirtuin variants. Our work summarized above raised questions how *C. elegans* SIR-2.4 and its mammalian homolog SIRT6 influence stress granule formation and cellular stress resistance.

Potential Molecular Mechanism of C. elegans SIR-2.4 in Stress Conditions

As previously shown, *C. elegans* SIR-2.4 is required for resistance to stressors such as heat shock through promoting DAF-16-dependent transcription and proper stress granules assembly.[27,28] The nuclear SIR-2.4 regulates DAF-16/FoxO acetylation indirectly, by preventing CBP-1-mediated acetylation under stress conditions.[28] It seems that an important function of SIR-2.4 is to promote DAF-16 nuclear recruitment and its transcriptional activity, and to control the expression of genes associated with aging and stress response such as SOD-3, HSP-16.1, DOD-3, DOD-24, INS-7.[28] Additionally, following heat shock, SIR-2.4 can be rapidly and reversibly redistributed into dynamic and cytoplasmic foci (granules) in the cytoplasm of somatic as well as of germline cells.[27] These data are consistent with our results regarding mammalian SIRT6 redistribution under stress response. In mammals, it is conceivable that a nuclear interaction between SIRT6 and G3BP modulates cytoplasmic functions of G3BP in stress granule assembly. This mechanism is due to dephosporylation of G3BP in a SIRT6-dependent manner in the nucleus that could alter G3BP function upon cytoplasmic translocation.[27] However, we cannot exclude that a minor fraction of SIRT6 present in secretory organelles, such as the endoplasmic reticulum,[31] might directly regulate G3BP function during cytoplasmic SGs assembly. Interestingly, sirtuin SIR-2.4 regulates DAF-16 function as well as stress granules assembly through other mechanisms than direct substrate modification.[27,28] Since we did not detect that SIRT6 deacetylates G3BP in vivo or in vitro we argue that SIRT6 did not direct modify G3BP. It has been speculated that SIRT6 can act as an NAD^+ metabolic sensor, binds NAD^+, and undergoes a conformational switch in the absence of substrate.[32] It seems likely that SIRT6 regulates components of the enzymatic machinery that controls the dephosphorylation status of G3BP. We can also not exclude that SIRT6 modifies additional proteins in the SGs multiprotein complex, and therefore, stabilizes the SIRT6-G3BP interaction, and impacts stress granules assembly via other mechanisms. It is possible that SIRT6 operates in the context of a chaperone complex (e.g., 14-3-3 or HSP-70) or microtubule network to promote the maturation of nuclear and cytoplasmic clients. SIRT6 might require chaperons for efficient activity. This interaction might stabilize an active conformation of SIRT6, and subsequently, stimulate protein deacetylation at the site of action. In turn, the enzymatic activity of SIRT6 can provide a rapid, economical, and reversible means to adjust protein functions during stress, thus affecting the survival as was observed.

It is surprising to observe that the nuclear protein SIRT6 accumulates in cytoplasmic SGs. Potentially, the nucleus undergoes a certain level of shutdown after stress to conserve energy and to sequester some nuclear proteins in cytoplasmic compartments thus providing a means for quick response to environmental changes. It is conceivable that the SIRT6 SGs function as quality control center to determine which proteins or RNAs need to be degraded, repaired, or stored. Deletion of SIRT6 or SIR-2.4 affects SGs formation, therefore including the possibility that SIRT6 have a regulatory role in the structure and dynamics of SGs. We argue that the recruitment of SIRT6 into SGs is not simply a result of non-specific protein aggregation. First, SIRT6 foci are reversible structures that disassemble immediately when stressors are replenished. Protein aggregation usually cannot be reversed in such a manner.[33,34] Second, the assembly and disassembly of SIRT6 SGs are regulated by the presence of stressors and also exhibited specific kinetics and patterns, which are uncharacteristic for non-specific protein aggregation.[35]

Concluding Remarks

Sirtuin family members have been implicated in aging and age-related diseases. Our studies reveal that SIRT6, in addition to its role in genomic stability, telomere protection, and modifications of histones, also regulates stress granules formation and cellular stress resistance through interplay with SGs components. By further dissecting the function of SIRT6 in SGs and its deacetylase activity in heat shock stressed cells, we may be able to uncover new pathways that cells use to regulate cellular stress response and lifespan.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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