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PARP-1 activation regulates recruitment of FUS to SGs and their assembly without additional recruitment of PAR to SGs

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Motivation and Aim: Synthesis of poly(ADP-ribose) (PAR) catalysed by PARP1 and PARP2 is one of the earliest events in the cellular response to DNA damage in higher eukaryotes. Upon binding to DNA breaks, PARPs catalyze the synthesis of PAR covalently attached to PARPs itself or an acceptor protein using NAD^+ as substrate for the post-translation modification [1]. PAR covalently attached to acceptor proteins can be subsequently hydrolysed by PAR glycohydrolase (PARG) thereby releasing free mono(ADP-ribose) or oligo(ADP-ribose) [2]. Recently, several reports have indicated that nuclear poly(ADP-ribose) polymerases (PARP1 and PARP2) play a critical role in the regulation of stress granules (SGs) assembly/disassembly [3–5]. SGs are ribonucleoprotein complexes containing untranslated mRNA and RNA-binding proteins (RBPs) that appear in the cytoplasm of mammalian cells upon the dissociation of polysomes, which occurs generally after various types of cellular stress [6]. There is real interest in deciphering the contribution of nuclear PARPs to SGs formation in cells under oxidative stress conditions, because SGs contain unstructured RBPs, such as TDP-43 and FUS that are associated with neurodegenerative diseases and can form insoluble cytoplasmic inclusions in the motor neurons of patients with amyotrophic lateral sclerosis [7]. We focused mainly on analysis of SGs recruitment of FUS (Fused in Sarcoma). FUS is one of the most abundant nuclear RBPs which interact with PAR and can be PARylated. In addition, we previously found that FUS is translocated to the cytoplasm after hydrogen peroxide treatment in a PARP1-dependent manner [8].
Methods and Algorithms: To analyze the formation of SGs and their composition under oxidative stress conditions we used HeLa cell line. The cells were treated with hydrogen peroxide and (or) arsenite, SGs formation in fixed HeLa cells was traced by immunofluorescence of protein/PAR components or in situ hybridization of cy3-labelled poly(dT) with poly(A)mRNA included into SGs. Statistical analysis of SGs number in cell or enrichment of proteins/PAR in SGs were performed using Cell Profiler software. PARP1 and PARP2 activities in cells were inhibited by chemical inhibitors (Olaparib or Talazoparib) or by using PARP1 siRNA. To analyze polysomes formation and translation regulation during PARP1 activation, we used polysome fractionation by sucrose density gradient centrifugation for comparison of polysome profiles in HeLa cells treated with arsenite and (or) hydrogen peroxide in the presence or absence of PARP1 inhibitor olaparib.
Results: By analysing SGs assembly in the cytoplasm of HeLa cells, we found that hydrogen peroxide, a potent nuclear PARP's activator, contrary to what might be expected, prevents SGs formation in the cytoplasm in a nuclear PARP1-dependent

manner [9]. Polysome profile analysis also showed that PARP1 activation upon DNA damage limits polysome dissociation, which is consistent with inhibition of SGs assembly. However, when SGs were formed in the cytoplasm, activation of nuclear PARPs positively regulates SGs production and makes them persistent in the cytoplasm. However, we failed to detect an increase of PAR enrichment in SGs, suggesting that PAR synthetized in the nucleus upon PARP1 activation is not recruited in SGs. This founding is very important for the function under stress conditions of many self-associated RNA-binding proteins that interact with PAR and/or undergo post-translational modification via poly(ADP-ribosyl)ation. Among them, FUS has been found to act in response to DNA damage through a PARP1-dependent mechanism [10]. In the present study, we show that PARP1 activation leads to FUS translocation from the nucleus to the cytoplasm and its accumulation in SGs of HeLa cells following hydrogen peroxide-induced oxidative stress, which in turn may promote SGs assembly, regulate an adapted translational response to stress and the aggregation of RNA-binding proteins in neurodegenerative diseases.

Conclusion: Overall, our data suggest that PARP1 activation after genotoxic stress can either block the formation of SGs if they were not preformed, but also can represent an aggravating factor in neurodegeneration diseases by preventing the dissociation of preformed FUS condensates.

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