Cell biology

Stress granules offer first aid for leaky organelles

Stephen P. Plassmeyer & Alex S. Holehouse

Assemblies of protein and RNA called stress granules appear in response to stressful conditions. The discovery that these granules can plug holes in punctured organelles sheds light on cellular defences against damage and infection. **See p.1062**

To a cell, stress corresponds to a perturbation that can affect growth and survival. In eukaryotes (organisms that include plants, animals and fungi), a common process associated with the onset of cellular stress is the formation of cytoplasmic assemblies made up of various proteins and RNA molecules, called stress granules[1]. These granules appear in response to a wide variety of stresses. Although their role remains a topic of debate, they seem to coordinate cellular reorganization to simultaneously protect normal 'housekeeping' programs, as well as facilitate emergency-response pathways[2,3]. On page 1062, Bussi *et al.*[4] report an additional and previously unappreciated role for stress granules in organelle repair: they can act as molecular plugs to patch up leaky lysosomes.

Lysosomes are specialized membrane-bound subcompartments found in animal cells. They function like stomachs, digesting components that enter the organelle. Digestive enzymes kept in the acidic interior of the lysosome break down and recycle cellular components, as well as dismantle bacteria and viruses that are internalized by the cell. If the lysosomal membrane were damaged by physical stress, chemical damage or invading disease-causing agents (pathogens), the release of these enzymes could degrade cellular components or trigger cell death.

The cellular response to lysosomal damage activates several membrane-repair mechanisms and triggers the formation of stress granules[5]. Together with the finding that essential stress-granule components (the proteins G3BP1 and G3BP2, collectively referred to as G3BP) associate with the lysosomal membrane even in the absence of stress[6], these observations have raised the question of whether stress granules contribute directly to the damage response.

To address this, Bussi *et al.* used high-resolution live-cell imaging to track the formation of stress granules after chemically induced lysosomal damage. The authors discovered that stress granules formed rapidly and specifically at the sites of damage (Fig. 1), appearing in seconds to form a 'plug' before infiltrating further into the lysosomal interior. Furthermore, in cells deficient in G3BP, the inability to form stress granules led to a decreased capacity to recover lysosomal integrity after acute damage, compared with that of cells with G3BP, suggesting that rapid lysosomal stabilization by stress granules is crucial for efficient membrane repair.

Stress granules are examples of biomolecular condensates, a type of membrane-less cellular assembly. The early stages of stress-granule formation are consistent with a process called phase separation, in which changes in biomolecular concentration or the cellular environment lead to the spontaneous condensation of molecules to form dense, protein-rich assemblies[6].

G3BP condensation can be triggered by several types of change in the environment. In some types of cellular stress, G3BP condensation is driven by a sudden increase in unbound cytosolic messenger RNA[7]. G3BP also undergoes condensation when pH drops[7], which seems to be the key trigger in stress-granule-mediated membrane-plug formation. Bussi *et al.* show that acidification of the lysosomal interior is necessary for damage-induced stress-granule formation and that a drop in pH across synthetic vesicle membranes could trigger G3BP1 condensation with RNA in an *in vitro* model.

The spontaneous and localized nature of stress-granule condensation makes it a well-suited mechanism for rapidly stabilizing membrane damage. However, Bussi *et al.* note that another characteristic of stress granules – membrane wetting – might further contribute to membrane stabilization and repair. Wetting refers to the ability of biomolecular condensates to spread over a surface. After the initial formation of a plug *in vitro*, G3PB1 condensates seem to spread along the surface of vesicle membranes.

To investigate the role that interactions between the membrane and stress granules might have, the authors used molecular simulations in which they could control interactions between modelled condensate-forming 'proteins' and the membrane. Condensate plugs could form without these interactions; however, attractive interactions between the condensate and membrane made this process more effective, and even seemed to promote membrane sealing. How, exactly, membrane wetting contributes to actual stress-granule plugging is unclear, but these theoretical results further motivate an exploration of the contribution of condensation as an efficient mechanism for membrane repair.

Is the spontaneous formation of biomolecular plugs a feature that is unique to stress granules? Probably not. Bussi *et al.* showed that a condensate-forming protein from soya bean could effectively plug vesicles *in vitro* in response to changes in pH and salt concentration across the membrane. Pioneering work in the early 2010s identified an entire class of proteins that lack a stable 3D structure (intrinsically disordered proteins) and that form biomolecular condensates and plug wounds in filamentous fungi[8]. There are also intriguing parallels between stress granules in this context and processes that occur during

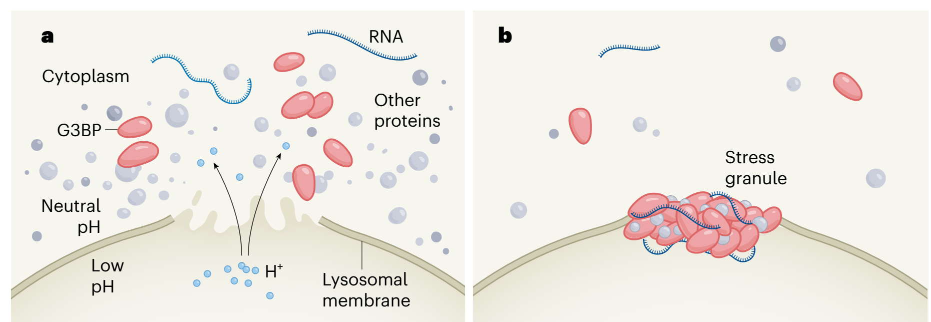

Figure 1 | Repair of membrane damage in lysosomal organelles. a, Damaged lysosomal membranes result in the exchange of contents between the lysosomal interior and the cytoplasm. Local changes in conditions around damage sites, such as pH changes driven by the movement of hydrogen (H^+) ions, alter the strength of interactions between the proteins G3BP1 and G3BP2 (collectively termed G3BP), and their interactions with other proteins and RNA. **b**, Bussi *et al.*[4] report that G3BP condenses at the damage site to form stress granules, which are collections of various proteins and RNA molecules. Stress granules prevent the further release of lysosomal contents and stabilize the membrane for the repair machinery.

the early stages of blood-clot formation – both involve the coordinated condensation of 'sticky biomolecules' (proteins and RNA for stress granules and cellular fragments called platelets for blood clots), albeit at different size scales[9].

Stress granules have been proposed to restrict viral infections[10], but their new-found role in stabilizing lysosomal membranes implicates them in defence against bacterial infection. The bacterium *Mycobacterium tuberculosis*, which causes the disease tuberculosis, damages lysosomes in host cells. This is central to the bacterium's ability to cause disease. In both cell and mouse models, Bussi and colleagues showed that *M. tuberculosis* infection resulted in the formation of lysosome-associated stress granules, which were absent from infections by a mutant *M. tuberculosis* strain with an impaired ability to damage membranes. Disrupting stress granules by G3BP depletion in human immune cells called macrophages greatly enhanced the replication of both types of *M. tuberculosis* strain. Given the key role that stress granules have in protecting against lysosomal damage, it might be expected that individuals with impaired stress-granule response pathways are more susceptible to tuberculosis infection.

Remarkably, the authors show that a previously identified marker[11] for tuberculosis susceptibility is a stress-granule protein that is recruited to stress granules that form near *M. tuberculosis* during infection. Overall, the authors' results simultaneously uncover a new role for stress granules in normal cellular function, and reveal their importance for host–bacterium interactions in the context of one of the world's most pressing global-health problems.

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In retrospect

15 years after a giant leap for cancer genomics

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In 2008, the first comprehensive sequence of a cancer genome was reported, ushering in a new era of molecular diagnostic, prognostic and therapeutic advances informed by an essential framework to understand cancer's complexities.

Efforts to understand the molecular underpinnings of cancer took a giant leap forward fifteen years ago, when Ley *et al.*[1] reported the first complete genome of a cancer – specifically, acute myeloid leukaemia (AML). Genomic data sets of this kind generated in the intervening years have revolutionized our knowledge about tumour biology.

A fundamental understanding of how cancer arises was established in the latter half of the twentieth century by scientists who studied the structures of chromosomes in diseased cells. Their efforts led to the discovery of an anomalous chromosome (termed the Philadelphia chromosome) that is associated with another type of leukaemia, chronic myeloid leukaemia (CML)[2]. This finding was followed by the discovery that this chromosome was formed by a type of exchange of parts of chromosomes called a reciprocal translocation[3]. In the 1990s, it emerged that this translocation generated a cancer-promoting protein called BCR–ABL, which is a fusion of components from two different proteins that forms a type of enzyme called a kinase. The kinase is unusual because it has continuous activity. The finding paved the way for the development and clinical application of a kinase inhibitor called imatinib, which inhibits BCR–ABL-mediated signalling[4]. This demonstrated that potent, specific targeting of a protein arising from a cancer-associated mutation was safe and could achieve durable remissions from cancer[5].

"The study by Ley *et al.* was a watershed moment in ushering in the modern era of cancer biology and therapy."

The success story of CML, once invariably fatal but now a disease that can be well controlled and sometimes eradicated by taking a pill, became an exemplar of precision medicine. At the turn of the twenty-first century, however, the field lacked a clearly defined framework to formulate a similar playbook for other cancers to fuel mechanism-based discovery and drug development.

In 2004, high-throughput sequencing of candidate genes uncovered key cancer 'driver' mutations, including those in the genes *PIK3CA* (ref. 6) and *EGFR* (refs 7,8). This success from a limited number of studies suggested that 'agnostic' studies – ones that do not focus solely on candidate genes but instead explore the entire cancer genome – would transform our understanding of the mutational repertoire of human cancers. In the wake of the Human Genome Project, completed in 2003, whole-genome sequencing of a tumour was within the realm of possibility. But there were considerable challenges to overcome, most notably the costs of sequencing cancer genomes, and the need for analytical tools to identify, at genome scale, somatic mutations (genetic changes that are not heritable because they occur in cells other than eggs or sperm).

It was in this context that Ley and colleagues took up the gauntlet to decode, at single-nucleotide resolution, the entire genome of cancer cells from a person with AML. The effort required a conviction that agnostic mutation discovery had the potential to spur new biological and therapeutic insights about cancer. It also necessitated rigorous foresight to devise analytical workflows that used genomic data derived from the individual's non-tumour cells, taken from the skin, to filter out mutations that were not relevant to the cancer – inherited variants unique to that person (germline polymorphisms) and 'passenger' mutations that occurred in the cancer but were not cancer-causing driver mutations (Fig. 1). Without paired cancer and normal data sets, it would have been impossible to comb through the 3.8 million variants in the cancer genome and identify the ten acquired driver mutations found by Ley *et al.*

The authors also developed a simple but