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Membrane-bound organelles *versus* membrane-less compartments and their control of anabolic pathways in Drosophila

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ABSTRACT

Classically, we think of cell compartmentalization as being achieved by membrane-bound organelles. It has nevertheless emerged that membrane-less assemblies also largely contribute to this compartmentalization. Here, we compare the characteristics of both types of compartmentalization in term of maintenance of functional identities. Furthermore, membrane less-compartments are critical for sustaining developmental and cell biological events as they control major metabolic pathways. We describe two examples related to this issue in Drosophila, the role of P-bodies in the translational control of *gurken* in the Drosophila oocyte, and the formation of Sec bodies upon amino-acid starvation in Drosophila cells.

1. Introduction

Cell compartmentalization is paramount for sustaining key cell biological events and programs of developmental biology. It is classically achieved by membrane-bound organelles, such as those forming the secretory pathway, the endosomal/lysosomal system, mitochondria, peroxisomes, lipid droplets, autophagosomes. It is nevertheless emerging that cell compartmentalization is also achieved by steady-state membrane-less assemblies in the nucleus, such as nucleoli, Cajal bodies and nuclear speckles, and in the cytoplasm, such as RNA based C. elegans P-granules, P-bodies, ribosomes, as well as others that do not contain RNA, like centrosome, proteasome and aggresome (Rajan et al., 2001).

Interestingly, the formation of many additional membrane-less assemblies (whether RNA based or not) is triggered by cellular stress, such as stress granules (Anderson and Kedersha, 2008; Protter and Parker, 2016); P-bodies that dramatically enlarge upon stress (Weidner

et al., 2014); DNA repair foci (Gibson and Kraus, 2012); Sec bodies (Zacharogianni et al., 2014; Aguilera-Gomez et al., 2016); higher order assemblies of metabolic enzymes in energy deprived yeast (Munder et al., 2016; Narayanaswamy et al., 2009; Petrovska et al., 2014; Riback et al., 2017), and A-bodies in the nucleus (Audas et al., 2016). Below, we introduce and compare both types of compartmentalization in terms of acquisition and maintenance of their functional identities. In the third part of this review, we describe how two Drosophila membrane less-compartments control anabolic pathways that are critical for developmental and cell biological events.

2. Membrane-bound organelles: maintaining their functional identity in the mist of intense membrane trafficking

With the exception of centrosomes and ribosomes, most cell

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biologists would consider the term organelle to be a functionally specialized unit within a cell that are separately enclosed within their own lipid bilayers (see Mellman and Warren (2000) for an overview). Membrane-bound organelles are a characteristic of eukaryotic cells. They are micrometer large, easily identifiable by microscopy and they can usually be isolated and/or purified by cell fractionation.

Organelle identity is defined by the presence of markers that often defines and carries the organelle function. These markers can be luminal, transmembrane and peripheral proteins. For instance, lysosomes contain cathepsins in their interior (Erickson, 1989), LAMPs integral to their membrane (Saftig and Klumperman, 2009), and Rab7 as a peripheral protein. The Golgi apparatus displays oligosaccharide transferases integral to its membrane (Fisher and Ungar, 2016; Gill et al., 2011) as well as numerous peripheral proteins, such as large coiled coil Golgins (Gillingham and Munro, 2016), GRASP65/55 (Rabouille and Linstedt, 2016) and COPI recruitment machinery (Jackson, 2014). ERESs are characterized by the concentration of the transmembrane protein Sec12, COPII subunits (Miller and Schekman, 2013) and Sec16 (Sprangers and Rabouille, 2015).

One important characteristic of cell organelles is that intensely communicate with one another. They do so by signaling (Bard and Chia, 2016; Farhan and Rabouille, 2011; Villasenor et al., 2016), but they also directly exchange materials *via* specific small vesicular carriers that transport cargo from organelle to organelle (Bonifacino and Glick, 2004; Spang, 2008). For example, COPII coated vesicles bud from the ER exit site (ERES) (Miller and Schekman, 2013), COPI coated vesicles bud mostly from the Golgi (Aguilera-Gomez and Rabouille, 2016; Jackson, 2014), and clathrin coated vesicles bud from the Trans Golgi Network (TGN) and the plasma membrane depending on the adaptors (Robinson, 2015).

Yet, despite this intense vesicular trafficking, the organelles maintain their functional identity. This is mostly achieved by ensuring that each coat binds the right membrane and that the fusion of transport vesicles with their target membrane is specific. Several classes of proteins including the small Rab GTPases (Barr, 2013), molecular tethers (Schroeter et al., 2016) and SNAREs (Malsam et al., 2008) ensure this specificity.

Furthermore, luminal and transmembrane markers that define organelle functional identity can be retained in their corresponding organelle. One clear example are Golgi enzymes (Banfield, 2011; Nilsson and Warren, 1994). Organelle markers can also be efficiently retrieved from un-correct locations. This is mediated by a number of signals in either their C-terminus (Nilsson and Warren, 1994; Pelham and Munro, 1993), such as the KDEL motif of luminal ER proteins (Munro and Pelham, 1987), and the KKXX motif for the ER transmembrane resident proteins (Nilsson et al., 1989). Indeed, when ER transmembrane proteins escape to the Golgi, their KKXX motif is recognized by, and encapsulated into COPI coated vesicles going back to the ER (Cosson et al., 1998; Ma and Goldberg, 2013). Therefore, the specificity of fission and fusion of transport vesicles is essential for the maintenance of organelle functional identities.

On the other hand, peripheral organelle markers are recruited to specific membrane organelle, often by Rab proteins. Their localization is further mediated coincidence detection by lipid binding/insertion and receptor binding. For instance, GRASP55 recruitment to Golgi membrane is Rab2 dependent and inserts in the membrane through its myristoyl moieties and its binding to Golgin 45 (Barr et al., 1998; Short et al., 2001). EEA1 is recruited to early endosomes through the binding of PI(3) *via* its FYVE domain (Stenmark et al., 1996) and Rab5 (Christoforidis et al., 1999).

3. Membrane-less compartments: formation and maintenance

Next to membrane-bound organelles, a number of cell compartments are membrane-less. They are also micron large multi-compo-

nent assemblies, contain many different types of biomolecules, and have specific functions. Examples range from the nucleolus, where ribosomes are made (Boisvert et al., 2007; Falahati et al., 2016); centrosomes that nucleate the interphase microtubule network (Mahen and Venkitaraman, 2012); nuclear Cajal bodies where spliceosomes are generated (Gall, 2003); P-bodies where basal RNA metabolism takes place (Parker and Sheth, 2007); P-granules that are essential for the development of the C. elegans embryo (Saha et al., 2016), and many RNA granules, especially those in the neurons that transport transcripts to the tip of neurites (Leung, 2014; Moser and Fritzler, 2010). As membrane-bound organelles, membrane-less compartments are also defined by specific set of markers, such as fibrillarin for the nucleolus, pericentrin and AKAP450 for centrosome (Bornens, 2002); DCP2, Ago and Me31B for P-bodies (Nakamura et al., 2001); PGL (germ granule components) and GLH (Vasa homologues) for P-granules (Updike and Strome, 2010).

In addition, many membrane-less compartments are formed during stress (Rabouille and Alberti, 2017). Importantly, most of these stress assemblies are reversible and may contribute to cell survival during stress and cell fitness upon stress relief. The best-studied stress assembly are stress granules that are formed upon accumulation of untranslated mRNAs caused by stress mediated eIF2 alpha phosphorylation resulting in protein translation inhibition (summarized in Aulas et al. (2017)). These mRNAs bind a number of RNA binding proteins (such as Tia1, G3BP, Caprin, dead box helicases, FUS) (Aulas et al., 2017) that coalesce to form meso-scale assemblies (Protter and Parker, 2016).

How are stress assembly components recruited and kept together in the absence of a membrane? An emerging concept in cellular organization is that membrane-less assemblies are formed through phase separation. Phase separation can be achieved through nuclear polymerization of one (or more) component that acts as a seed to form solid/crystalline assemblies. This can be driven by changes in metabolites concentrations or pH changes combined with molecular crowding, such as in yeast upon energy depletion (Munder et al., 2016; Rabouille and Alberti, 2017). Phase separation can also be mediated through liquid-liquid phase transition (Hyman and Simons, 2012; Weber and Brangwynne, 2012) resulting in liquid droplet formation that can "age" into gels or glass (at least *in vitro* (Patel et al., 2015)). Both create compartments that are enriched in pools of active biomolecules while others are repelled (Su et al., 2016). Furthermore, these compartments can function as storage, protection and a controlled release mechanism for macromolecules.

Liquid-liquid phase separation defines the behavior of an initially homogeneous solution of macromolecules that demixes into two distinct liquid phases that then stably coexist (Brangwynne et al., 2009; Hyman et al., 2014). This behavior underlies the formation of stress granules (Protter and Parker, 2016). Liquid droplets (*in vivo* and *in vitro*) are mostly spherical; the molecules within them can freely diffuse; liquid droplets fuse easily to relax their surface tension; and critically they are reversible, for instance upon stress relief.

Liquid-liquid phase separation is usually triggered by an increase in local concentration of certain types of proteins that display a number of features: i) They are often multivalent (used to define the number of possible interactions that each molecule has); ii) They are flexible and have the propensity to adopt a large range of conformations. These proteins often display intrinsically disordered domains that have low complexity sequences (Huntley and Golding, 2002). Low complexity sequences are regions of poor amino-acid diversity, such as repeats of certain amino-acids (Q, N, S, G, Y, R) in prion-like domains (Alberti et al., 2009), repeats of alternating charges, such as RG, and other domains without regular sequences.

The enrichment in charged amino-acids in low complexity sequences suggest that electrostatic interactions play an important role in liquid-liquid phase separation. The best evidence comes from *in vitro* studies using purified proteins with low complexity regions, such as dead box helicases Ddx4 (Nott et al., 2015) and Ddx3 (Elbaum-Garfinkle et al.,

2015), RNA binding proteins, Fus, hnRNPA1, Tia-1 (Lin et al., 2015; Molliex et al., 2015; Patel et al., 2015) and Pab1 (Riback et al., 2017). These purified proteins phase separate *in vitro* to form single component liquid droplets (recently called quinary droplets (Kroschwald and Alberti, 2017)). In addition to depending on the presence of these low complexity sequences, phase separation is also strongly dependent on the ionic strength (Molliex et al., 2015; Nott et al., 2015) and pH (Riback et al., 2017). In addition, hydrophobic interactions (Riback et al., 2017) and short-range interactions, such as dipole-dipole (between polar amino-acids), pi-pi (aromatic amino-acids) and cation-pi (interaction between aromatic and basic residues) also appear to play an important role (Banani et al., 2017; Brangwynne, 2015).

RNA increases phase separation of RNA binding proteins *in vitro* (Lin et al., 2015; Patel et al., 2015) and *in vivo* (Molliex et al., 2015). Increase concentration of mRNAs therefore appear to be the critical determinant for the formation of membrane-less assemblies. However, this has recently been challenged with the demonstration that Pab1 phase separation is inhibited by the presence of RNA. This leads to the interesting hypothesis that the formation of small Pab1 quinary droplets *in vivo* would free mRNA encoding key stress response factors, thus enabling cells to cope with heat stress and energy deprivation. Indeed, when Pab1 gelation is compromised, stressed yeast die (Kroschwald and Alberti, 2017; Riback et al., 2017).

Last, it is also clear that stress specific post-translational modifications promote the formation of membrane-less compartments *in vivo* and phase separation *in vitro* (Han et al., 2012; Kato et al., 2012). This is case for SUMOylation and phosphorylation (Banani et al., 2016), ubiquitination for proteasome storage granules (Peters et al., 2013), poly-ADP ribosylation (Leung et al., 2011) and mono-ADP-ribosylation (Aguilera-Gomez et al., 2016). Conversely, arginine methylation by PRMT1 has been shown to be inhibitory (Jun et al., 2017; Nott et al., 2015), and phosphorylation by DYRK3 leads to stress granule dissolution (Wippich et al., 2013).

Do membrane less-assemblies communicate with other parts of the cell? First, a number of stress assemblies are formed in response to signaling elicited by stress, such as stress granules, Sec bodies (see below) and A bodies (Audas et al., 2016). Conversely, stress granules have been proposed to modulate signaling pathways, such as apoptosis by sequestering key pro-apoptotic components (Arimoto et al., 2008; Kim et al., 2005) and the TOR pathway (Takahara and Maeda, 2012; Thedieck et al., 2013; Wippich et al., 2013). Second, P-bodies and stress granules communicate. mRNAs that are stored in stress granule can be sorted in P-bodies for degradation if they are damaged (Buchan et al., 2008) (Buchan and Parker, 2009; Kedersha et al., 2005) and both structures can be found in close proximity (Souquere et al., 2009). Third, membrane-less compartments communicate with membrane-bound organelles. For instance, yeast P-bodies communicate with the ER that modulate their formation (Weidner et al., 2014). Furthermore, membrane-less assemblies can form in the lumen of an organelle. This is the case of GPI anchored protein PrP based structures that form at the TGN and endosomes in yeast (Ritz et al., 2014).

How is membrane-less assemblies communicate with the rest of the cells remains to be better investigated. It is likely controlled by the controlled diffusion and incorporation of their components as it is the case of damaged RNAs between stress granule and P-bodies, and the active shuttling of TIA-1 from stress granules to polysomes in order to selectively enhance or repress mRNA expression (Kedersha et al., 1999). Last, as mentioned above, many stress assemblies are the result of activated stress signaling pathways, but do they initiate signaling by recruiting and activating, for instances kinases and GTPases?

4. Similarities and differences between membrane-bound and membrane-less compartments

The first difference is obviously the presence of a membrane. This is not only a physical barrier that regulates the exchange between the cytoplasm and the lumen, but it also acts as a platform for recruitment of peripheral proteins (see above) that may control signaling pathways. Nevertheless, the absence of a membrane does not mean unregulated exchange between the core of a membrane-less compartment and the surrounding space. It is not mediated by membrane transporters but by the strength of interactions between the components. These interactions not only regulate component release but also prevents others with less affinity to reach the inside.

The second difference is their biogenesis. At least nuclear membrane-less compartments appear to form de novo after their disassembly at early mitosis (Dundr and Misteli, 2010). Although some membrane-less compartments, such as peroxisomes (Agrawal and Subramani, 2016) and lipid droplets (Wilfling et al., 2014) can also form de novo, many membrane-bound organelles are not. For instance, during mitosis, most of the organelles undergo fission (sometimes leading to severe fragmentation) that generates templates onto which membrane and protein components are recruited. This follows the principle of self-organization that involves the physical interaction of molecules to form a steady-state structure (Prigogine et al., 1974; Misteli, 2001). This is not to be confused with self-assembly that involves the physical association of molecules into an equilibrium structure (Misteli, 2001), such as those existing in viruses. Self-organization is also what underlay the formation of membrane-less assemblies that are steady-state structures rather than structures at equilibrium. This is not only true for the structures that are formed under stress and that are rapidly dissolve upon stress relief, but also for those that exist in basal conditions, such as nucleolus (Lewis and Tollervey, 2000) and the Cajal bodies (Dundr et al., 2004) that constantly and rapidly exchange molecules with the nucleoplasm.

The third difference is the time scale of their response to (environmental) signaling/perturbations. Perhaps the most dramatic difference is the formation within minutes of stress assemblies from seemingly diffuse (or nanoscopic) components upon stress signaling, as well as their dissolution upon stress relief, as opposed to the rather stable presence of membrane-bound organelles. However, this difference is perhaps not as strong as suggested. Indeed, addition of drugs, such as Brefeldin A, leads to the re-absorption of the Golgi stacks in the ER in a matter of minutes in most mammalian cells (Lippincott-Schwartz et al., 1989), and stress also leads to rapid changes in its architecture (Cancino et al., 2013; Farhan and Rabouille, 2011).

The fourth difference is the type of proteins that are found in these two types of compartments. As mentioned above, membrane-less compartments appear to be enriched of intrinsically disordered proteins containing low complexity sequences whose properties mediate their formation. This does not appear to be the case for membrane-bound organelles but these proteins have not yet been studied enough in this context and they might also be represented in the organelle proteomes. For instance Sec16, Sec24 and GRASP65/55 have a high content of low complexity sequences (Zacharogianni et al., 2014). Furthermore, GPI-anchored prion-like proteins act as retention mechanism in the yeast TGN through their formation of small aggregates (Ritz et al., 2014).

Overall, the similarities between membrane-bound and membrane-less compartments may outweigh their differences. In this regard, membrane-less compartments, as their membrane-bound counterparts, are also known to control anabolic pathways. This is the case for the production of ribosomes and spliceosomes in the nucleolus and Cajal bodies, respectively and below, we present further illustrations of this notion with implications in developmental and cell biology.

5. Membrane-less compartments and anabolic pathways

5.1. P-bodies and translational control in the developing Drosophila oocyte

5.1.1. Translation in compartmentalized P-bodies

As mentioned above, P-bodies are the crucible in which most of the

RNA metabolism takes place, including degradation through endonuclease activity, decapping, RNAi mediated pathway as well as microRNA mediated degradation (Parker and Sheth, 2007).

However, P-bodies can also store translationally silent mRNAs (Aizer et al., 2014). In this respect, attention has been drawn to their role in translational control during plant development (Xu and Chua, 2011) and Drosophila oogenesis (Snee and Macdonald, 2009; Weil et al., 2012). There, oocyte P-bodies were shown to be key for the targeted localization and translation control of the developmentally critical mRNAs gurken and bicoid (Delanoue et al., 2007; Weil et al., 2012). Of note, oocyte P-bodies were first described as sponge bodies (so named because of their appearance) (Delanoue et al., 2007; Nakamura et al., 2001; Wilsch-Brauninger et al., 1997) but were further shown to contain many translational control components (Snee and Macdonald, 2009; Weil et al., 2012).

The oocyte P-bodies largely mediate the localization of both gurken mRNA at the oocyte dorsal anterior corner at stage 7–9, and of bicoid mRNA around the entire anterior cortex (Johnston et al., 1989). gurken and bicoid transcripts are even localized to the same P-bodies at the dorsal anterior corner. However, gurken tends to be enriched at the edge of these structures whereas bicoid in enriched in their core, reflecting a potential functional sub-compartmentalization of the P-bodies (Weil et al., 2012).

The P-body core is further defined by the absence of ribosomes and the enrichment of translational repressors, whereas their edge is enriched in Orb and ribosomes are present. The edge of oocyte P-bodies is therefore proposed to be translational active whereas their core is translationally silent. Interestingly, this organization mirrors the translational status of both mRNAs. gurken mRNA, at the P-body edge is translated at stage 8–9, whereas bicoid mRNA is stored in their core and will only be translated at stage 14 of oogenesis (Weil et al., 2012) (Fig. 1).

5.1.2. Orb modulates gurken translation

Does P-body sub-compartmentalization explain why gurken RNA is not translated in the adjacent nurse cells where it is transcribed? Indeed, gurken mRNA is synthesized in the nurse cell nucleus (Caceres and Nilson, 2005). It is then transported through ring canals along microtubules to the dorsal anterior corner of the oocyte (Clark et al., 2007) where it is anchored to oocyte P-bodies and translated (Delanoue et al., 2007; Weil et al., 2012). However, what prevents it to be translated in the nurse cells was till recently not clear.

There were two possibilities: gurken mRNA molecules could be bound to repressors and buried in the core of the nurse cell P-bodies, an hypothesis sustained by the identification of gurken repressors, such as Bruno (Filardo and Ephrussi, 2003; Reveal et al., 2011; Yan and Macdonald, 2004), and the dead end helicase Me31B (Kugler et al., 2009; Nakamura et al., 2001). Alternatively, the composition of the nurse cell P-bodies could be different from that of oocyte P-bodies and unable to support gurken translation.

The MS2 system (Bertrand et al., 1998; Jaramillo et al., 2008) was used to visualize gurken mRNA dynamics in the nurse cells of the Drosophila egg chamber, and it showed that gurken mRNA does not associate to P-bodies, either in their core or at their edge (Davidson et al., 2016). This suggests that gurken transcripts are not able to anchor to the nurse cell P-bodies. This inability to anchor was investigated by assessing whether proteins normally associated to the oocyte P-bodies were also present in the nurse cell structures. The most critical difference was that Orb level was significantly lower in the nurse cells and was absent from nurse cell P-bodies (Davidson et al., 2016). (Fig.1)

Orb is the Drosophila homologue of cytoplasmic polyadenylation element binding protein (CPEB) that is a key translational activator of gurken. Consequently, gurken is not translated in an Orb mutant (Chang et al., 2001). Furthermore, Orb forms a complex with poly(A) polymerase Wispy and this is required for grk hyper-adenylation as
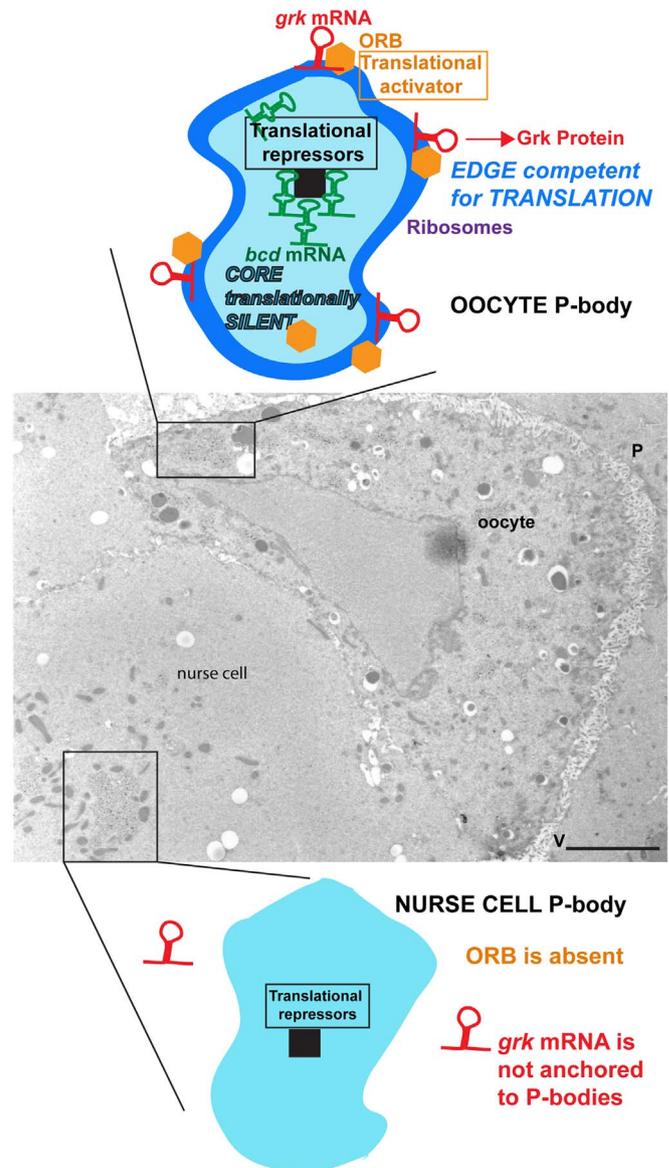
Fig. 1. Orb control of gurken translation at the edge of the Drosophila oocyte P-bodies. Electron micrograph of a frozen ultrathin section of stage 8/9 Drosophila egg chamber labeled for Me31B (10 nm gold) to mark P-bodies. Note that the labeling is similarly restricted to P-bodies in the oocyte and the nurse cells (examples are boxed). Scale bar: 10 µm. The schematics above the micrograph represents a sub-compartmentalized oocyte P-body at the dorsal-anterior corner with its translational silent core containing bicoid mRNA and its translational competent edge to which gurken mRNA, Orb and ribosomes are associated, thus allowing Gurken synthesis. The schematics below the micrograph represents a nurse cell P-body to which gurken mRNA is not associated due to the absence of Orb. As a result, it is not translated. Hence, what controls gurken translation is Orb association to P-body edge.

well as its translation (Norvell et al., 2015; Wong et al., 2011). To test whether Orb is the key factor regulating gurken translation, it was overexpressed in the nurse cells using two genetic systems, both resulting in the clear ectopic expression of Gurken protein in these cells. Critically, upon Orb expression, gurken mRNA was now associated to nurse cells P-bodies together with Orb (Davidson et al., 2016). This indicates that Orb is one determining factor controlling gurken anchoring to P-bodies where it is translated. Where Orb is absent or low, gurken mRNA is not anchored and not translated (Davidson et al., 2016; Derrick and Weil, 2016).

Taken together, this shows a critical role for the composition and sub-compartmentalization of the membrane-less assembly P-body in the control of gurken translation.

5.2. Sec bodies and protein transport in the early secretory pathway

5.2.1. Sec bodies form to protect ERES components

Next to protein translation, protein transport through the secretory pathway is another major anabolic pathway. This pathway is formed by a series of morphologically and functionally defined membrane-bound compartments. It is used to deliver proteins to the plasma membrane, all membrane compartments (except mitochondria), and extracellular medium. Newly synthesized proteins exit the ER in COPII coated vesicles at the ER exit sites (ERES). COPII coat vesicles formation starts by the Sec12 mediated conversion of Sar1-GDP to its GTP form and its recruitment to ER membrane. This is followed by the recruitment of the heterodimer Sec23/24 forming the inner layer of the coat (Antonny et al., 2001; Bi et al., 2007; Lord et al., 2011) that in turn recruit the dimer Sec13/31 forming the outer layer (Stagg et al., 2008). A factor necessary for COPII dynamics is the large ERES scaffold protein Sec16 (Kaiser and Schekman, 1990; Sprangers and Rabouille, 2015) that concentrates Sar1 and negatively regulates the uncoating of the bud (Sprangers and Rabouille, 2015). Newly synthesized proteins reach the Golgi apparatus where they are processed, sorted and dispatched to their correct cellular localization. Together, the ERES and the Golgi form the early secretory pathway.

Cellular stress, such as heat stress (Petrosyan and Cheng, 2014), oxidative stress, and genotoxic stress leading to DNA damage (Farber-Katz et al., 2014) leads to an inhibition of protein transport and affects the functional organization of the early secretory pathway, especially the Golgi apparatus. In Drosophila cells, the stress of amino-acid starvation also inhibits protein transport through the secretory pathway (Zacharogianni et al., 2011) and leads to the remodeling of the ERES components into a novel membrane-less stress assembly, the Sec body (Zacharogianni et al., 2014). During the period of stress, Sec bodies store and protect most of the COPII components and Sec16 from degradation. They are round and display FRAP properties compatible with having liquid droplet properties. Importantly, they are pro-survival and rapidly disassemble upon stress relief. When stress is relieved, Sec bodies rapidly dissolve releasing their functional components that resume protein transport (Zacharogianni et al., 2014). As such, Sec bodies are part of the adaptive response to stress (Fig. 2).

Interestingly, amino-acid starvation also leads to the formation of another stress assemblies, the stress granules that form in response to the inhibition/stalling of protein synthesis elicited by the stress (Zacharogianni et al., 2014). Both Sec bodies and stress granules form close to one another in S2 cells but they do not mix. Whereas Sec bodies protect COPII components and Sec16 (not seemingly no mRNA), stress granules are mRNA and translation machinery rich structures (Fig. 2).

5.2.2. Sec16 mono-ADP-ribosylation by ER-localized dPARP16 is required for Sec body formation

As mentioned in Part I, the formation of membrane-less compartments is often triggered by stress (Rabouille and Alberti, 2017). But how is the stress sensed by the cell interior to lead to the formation of Sec bodies? What happens to Sec16 and COPII subunits upon the stress od amino-acid starvation? The traditional view is that stress stimulates signaling pathways that transmit environmental fluctuations to the cell interior, for instance by promoting post-translational modifications. In this regard, Leung and colleagues (Leung et al., 2012) have proposed that protein Poly-ADP-ribosylation (PARylation) (consisting in the addition of two or more ADP-ribose units to a protein substrate by ADP-ribosyltransferases (PARPs) (Gibson and Kraus, 2012; Hottiger et al., 2010)) triggers events leading to liquid-liquid phase separation (Protter and Parker, 2016).

The funding member of ADP-ribosylation, the nuclear PARP1 is required for DNA repair during DNA damage (Gibson and Kraus, 2012; Jungmichel et al., 2013) where it hyper-PARylates itself as well as
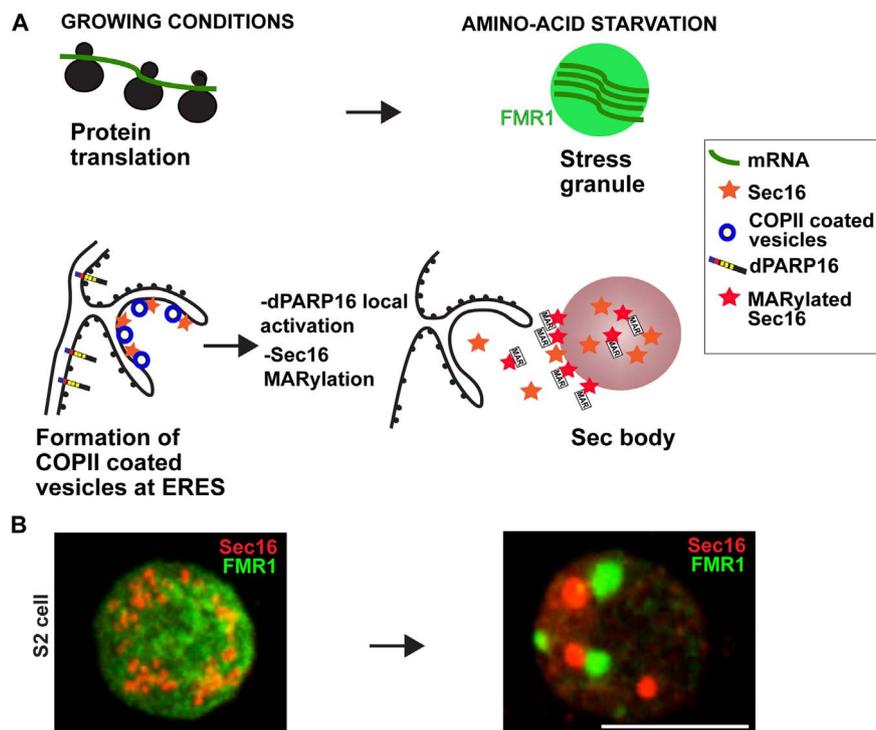
Fig. 2. The amino-acid stress response in Drosophila S2 cells. A: Schematics of protein translation and protein transport out of the ER at ER exit sites (ERES, where COPII coated vesicles formed) that are both active in growing conditions. Upon amino-acid starvation, translation initiation is inhibited. Untranslated mRNAs accumulate and are bound by RNA binding proteins, such as FMR1, leading to the formation of membrane-less stress granules. Amino-acid starvation also inhibits protein transport out of the ER. ER localized dPARP16 is activated and MARylates Sec16 on its C-terminus leading to the formation of Sec bodies that protect COPII components and Sec16 from degradation. **B:** Immunofluorescence visualization of FMR1 (green) and Sec16 (red) in growing cells. FMR1 is cytoplasmic and Sec16 is localized at the ERES. Amino-acid starvation leads to the formation of stress granules (marked by FMR1) and Sec bodies (marked by Sec16). Scale bar: 10 μm.

surrounding histones (Gibbs-Seymour et al., 2016). PARylation is also associated with stress granule formation in mammalian cells by facilitating the local concentration of proteins and promoting their oligomerization (Leung, 2014). Evidences range from the fact that stress granule components are PARylated upon sodium arsenite treatment (Leung et al., 2011). Stress granules contain PARPs, especially PARP13 (Leung et al., 2011, 2012) and PARP12 (Welsby et al., 2014). Overexpression of these PARPs induces stress granule formation, even in the absence of stress. Furthermore, the PARylation inhibitor 3-aminobenzamide completely inhibits stress granule formation (Leung et al., 2011). Last, stress granules also contain poly(ADP-ribose) glycohydrolase (PARG) that hydrolyses the poly-ADP-ribose chains (Hatakeyama et al., 1986), and its overexpression also inhibits their formation (Gagne et al., 2005; Leung et al., 2012). Together, this has led to the model that stress-induced PARylation of RNA binding proteins leads to their coalescence into cytoplasmic assemblies containing RNAs, PARPs and PARGs (Leung, 2014).

In line with this, we have found Sec body formation requires Mono-ADP-ribosylation (MARylation, addition of a single ADP-ribose) by the ER localized dPARP16 that is activated by amino-acid starvation (Aguilera-Gomez et al., 2016). dPARP16 over-expression drives Sec body formation even in the absence of stress. Conversely, dPARP16 depletion upon amino-acid starvation prevents their formation. dPARP16 is expressed at very low level in Drosophila cells but when depleted, cells are more sensitive to amino-acid starvation and they do not recover well after stress relief. As such, dPARP16 is a novel stress survival factor.

Interestingly, using a probe that we designed to specifically detect MARylation events *in vivo* (Aguilera-Gomez et al., 2016), we showed that amino-acid starvation triggers a strong wave of cytoplasmic MARylation events that take place in the same time frame as Sec body formation and localizes in close proximity to these assemblies. Using this probe, the Sec body component Sec16 has been demonstrated to be one of the dPARP16 substrates upon amino-acid starvation, and we identified the small sequence in the Sec16 C-terminus that is MARylated. Altogether, dPARP16 dependent Sec16 MARylation is necessary and sufficient for Sec body assembly (Aguilera-Gomez et al., 2016).

These findings show that MARylation of one substrate by a specific enzyme can drive a major remodeling of the cellular architecture. Moreover, this finding not only establishes an unprecedented role for MARylation in the formation of membrane-less assemblies, but also links this post-translational modification to a specific metabolic stress, to membrane traffic in the early secretory pathway, and to cell survival pathway.

6. Conclusion and perspectives

The field of membrane-bound organelles has been intensely studied during the last 50 years and they are fairly well understood. However, new fields are emerging, such as the role of membrane contact sites (Gatta and Levine, 2016), for instance in the formation of sub-organelle structures like mitochondrial cristae (van der Laan et al., 2016); the role of membrane-bound compartments as signaling platforms, receiving and sending signals (Cancino et al., 2013; Farhan and Rabouille, 2011); their functional and morphological response to stress (for instance Golgi stress (Machamer, 2015)); and the identification of new roles for known molecules and pathways, such as Arf1 in mitochondria morphology (Ackema et al., 2014) and O-linked glycosylation (Bard and Chia, 2016; Pompa et al., 2017).

Membrane-less compartments have also been described for a long time but they only recently have become the focus on intense research in terms of their dynamics (formation, maintenance and dissolution), composition, and their role in stress survival. Furthermore, as described above, specific biochemical reactions are uniquely taking place in these compartments and this will need to be further investigated.

Last, their de-regulation could have deleterious consequences. For instance, it is now clear that faulty stress granule dynamics is linked to neurodegeneration of motor neurons (ALS) (Li et al., 2013; Molliex et al., 2015; Patel et al., 2015) where mutant stress granule components induce the formation of irreversible fibrous aggregates.

Finally, it will be interesting to understand better how membrane-bound and membrane-less compartments interact with one another. It seems for instance that P-bodies are assembled at the surface of the ER in yeast, thus presumably controlling translation there (Weidner et al., 2014). Is it the case for other membrane-less compartments? In this regard, it should be noted that prion-based membrane-less small aggregates appears to form in the lumen of the yeast TGN and mediate retention of a specific class of proteins (Ritz et al., 2014). Last, Sec16, a protein of the early secretory pathway is a key factor required for Sec body formation. How the biology of membrane-bound organelles and membrane-less compartments is linked will be the next challenge.

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