Stress-induced protein aggregates shape population heterogeneity in bacteria

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Abstract

The concept of phenotypic heterogeneity that prepares a subpopulation of isogenic cells to better cope with anticipated stresses has been well-established. However, less is known about how stress itself can drive subsequent cellular individualization in clonal populations. In this perspective, we focus on the impact of stress-induced cellular protein aggregates, and how their segregation and disaggregation can act as a deterministic incentive for heterogeneity in the population emerging from a stressed ancestor.

Bacteria typically proliferate asexually by binary fission, and thereby tend to yield clonal populations of isogenic siblings. Nevertheless, despite an identical genetic make-up and environment, such siblings can have their own individuality in terms of molecular composition, gene expression and, eventually, phenotypic behavior (Ackermann, 2015; Davis and Isberg, 2016). In fact, evolution might positively select for the emergence of phenotypic heterogeneity as it enables complex social behavior among siblings that favors adaptation and survival of the population as a whole (Kussell and Leibler, 2005; Veening *et al.*, 2008; Ackermann, 2015; West and Cooper, 2016).

Indeed, phenotypic heterogeneity can enable bet-hedging strategies for clonal populations in fluctuating environmental conditions by expressing protective or adaptive features in only a subset of individuals. This subpopulation is therefore pre-adapted to and better able to survive an anticipated (and perhaps sudden) change in environment for which the more conventional sense-and-respond approaches may not be adequate. However, since the expression of these features tends to be costly and compromise fitness when it is not matched with the environment, it is not exhibited by the entire population. One of the best-documented examples of bet-hedging is the phenomenon of persistence, in which a subpopulation of cells is in a transient growth-arrested state that confers tolerance to antibiotics (Gefen and Balaban, 2009). While this fraction of cells does not contribute to the growth of the population, they are more likely to survive an episode of antibiotic stress and become the founders of the new population thereafter. Another benefit of phenotypic heterogeneity is its potential to serve as a division of labor strategy, in which a certain task is performed by only a fraction of the siblings, but nevertheless benefits the population as a whole. An example of this is the production of the secreted protease subtilisin E in *Bacillus subtilis.* The protease is only produced by a

subset of cells, but its secretion results in diffusible degradation products that are accessible for all cells of the population (Veening *et al.*, 2008). The entire population is therefore expected to benefit from taking up these degradation products, while the associated cost of producing them is limited to only a fraction of cells. Population heterogeneity can in fact also combine both strategies, as was shown for virulence gene expression in *Salmonella* Typhimurium. In this case, a subpopulation that is slow-growing because of the production of virulence factors (*i*) takes on the labor to instigate host gut inflammation that enables effective host colonization by the rest of the population, but (*ii*) also displays increased tolerance to antibiotics (Arnoldini *et al.*, 2014).

Interestingly, several molecular mechanisms have been identified as a source for individualization of isogenic siblings, which could in turn result in phenotypic heterogeneity. The initial driver for individualization is often stochastic in nature, such as random partitioning of small numbers of molecules among progeny or intrinsic noise in gene expression (Elowitz *et al.*, 2002; Huh and Paulsson, 2011; Bidnenko and Bidnenko, 2018; Evans and Ling, 2018). Such stochastic differences can themselves already have biologically relevant phenotypic repercussions. In this context, it was shown for *Escherichia coli* that phenotypic behavior of sister cells (with respect to heat shock survival) tends to be similar for a brief time after cell division, but rapidly becomes randomized thereafter, likely due to a combination of multiple stochastic cellular processes (Govers *et al.*, 2017). Also in *E. coli,* it has been suggested that transition towards persistence is mediated by stochastic fluctuations in HipA toxin expression, which in turn causes growth arrest and antibiotic tolerance when HipA levels exceed a threshold (Rotem *et al.*, 2010). In some cases, however, genetic circuitry can deliberately amplify such initial stochastic differences into dedicated and often stably inheritable gene

expression patterns, with the consequent emergence of differentiated subpopulations (Smits et al., 2006; Casadesús and Low, 2013). Often, these are governed by positive (or double-negative) feedback loops, as is the case for lactose utilization through the *lac*-operon in *E. coli* (Novick and Weiner, 1957; Ozbudak *et al.*, 2004; Robert *et al.*, 2010) and natural competence in *B. subtilis* (Smits *et al.*, 2005).

However, more deterministic cellular processes can instigate cellular individuality as well. A recent study in *E. coli*, for example, showed that the inheritance of the old cell pole and the presumed associated asymmetric accumulation of damage results in predictable heterogeneous age structures and associated growth rate variabilities (Proenca *et al.*, 2018). Similarly, the main multidrug efflux pump of *E. coli* (i.e. AcrAB-TolC) displays strongly biased partitioning towards the old pole, resulting in increased drug efflux activity and fitness under subinhibitory drug concentrations of old-pole inheriting cells (Bergmiller *et al.*, 2017). Although not as widely studied as genetic circuitry, these examples demonstrate that asymmetric partitioning of molecular structures can provide potent and deterministic drivers of heterogeneity.

In some cases, population heterogeneity is not present by default but can be triggered and tuned by environmental conditions. In *Klebsiella oxytoca*, for example, the availability of NH_4^+ (as the more preferred N-source) shapes phenotypic heterogeneity in N_2-fixation (i.e. the energetically costly conversion of N_2 into NH_4^+) (Schreiber *et al.*, 2016). When NH_4^+ becomes limited, cells will increase phenotypic heterogeneity in N_2-fixation, while simultaneously still engaging in NH_4^+-uptake. This heterogeneity in N_2-fixation during mixed N-source conditions (NH_4^+ and N_2) allows the population to better prepare for N-source fluctuations, as the subset of cells with high N_2-fixation have increased growth

rates when an environmental switch to NH_4^+-depletion occurs. Another notable example in this category is the process of sporulation, where starvation stress triggers the differentiation of two sister cells into a mother and a forespore cell, which eventually culminates in the formation of a recalcitrant endospore (Stragier and Losick, 1996). Additionally, the environmental cue of nutrient limitation is sensed by a noisy multicomponent phosphorelay, which confers heterogeneous entry into sporulation by phosphorylation of the master regulator Spo0A (Russell *et al.*, 2017), and differentiates the population into functionally different subpopulations of sporulating and non-sporulating cells (Veening *et al.*, 2008).

A more recent example of how the perception of stress can serve as a decisive event that itself triggers the emergence of cellular individualization and population heterogeneity concerns the formation of protein aggregates (PAs) in *E. coli*. Denatured proteins that emerge as a result of proteotoxic stress tend to coalesce into relatively large intracellular PA structures that become randomly allocated to one of the cell poles by nucleoid occlusion (Lindner *et al.*, 2008a; Winkler *et al.*, 2010; Coquel *et al.*, 2013; Govers et al., 2014; Govers *et al.*, 2018). Upon survival and outgrowth of the stressed cell, the subsequent inevitable asymmetric segregation of this undividable structure then deterministically creates a heterogeneous population in which some cells inherit the ancestral PA, while others do not (Fig. 1A). In contrast to the bad reputation of PAs with respect to cellular fitness (Ross and Poirier, 2004; Lindner *et al.*, 2008b), it was found that the presence of such an ancestral PA improved cellular robustness upon encountering a subsequent proteotoxic stress (such as heat, hydrogen peroxide and streptomycin) (Govers *et al.*, 2018). This indicates that PAs may not be simple 'garbage bins' of damaged proteins but can serve as a type of memory that persists through multiple generations

after formation by a previous (ancestral) stress. As such, PAs somewhat resemble prion-based memory (Gasset-Rosa *et al.*, 2014; Chernova et al., 2017). However, PAs are not self-proliferative and likely consist of a multitude of different protein species, making it difficult to attribute PA-based phenotypes to the loss-of-function of a given protein. In fact, it is currently hypothesized that PA-mediated memory is accomplished by the specific enrichment of protein quality control elements (such as DnaK, DnaJ, ClpB, IbpA and ClpP) due to co-localization and co-inheritance with the PA (Govers *et al.*, 2018).

In further contrast to self-proliferating prions, PA's seem to be continuously disaggregated, presumably because of this close association of quality control proteins with the PA. In fact, with a fluorescently labelled PA, this disaggregation even becomes evident as a PA-derived fluorescent "trail" or "wake" in the siblings spawning off from the PA carrier cell (Fig. 2A, left panel). As a result of disaggregation and subsequent dilution by cell growth and division, the concentration of disaggregated proteins in these siblings is correlated with their degree of kinship to the PA-bearing cells (Fig. 2B). When deleting the *dnaK* gene, thereby blocking the DnaKJE chaperone system that is known to play an important role in disaggregation (Doyle et al., 2013), the generation of this wake is completely abolished (Fig. 2A, right panel). This indicates that the establishment of this wake is not an artefact of the fluorescent model system but a true biological consequence of cellular PA management.

Although this remains subject to further study, it is tempting to think that (aside the actual PA) this disaggregation-borne trail of degraded and refolded proteins could constitute another driver of intercellular heterogeneity within the lineage emerging from a proteotoxically insulted cell (Fig. 1A and B). Obviously, it still remains to be discovered

to which extent natural PAs would be subjected to the same disaggregation dynamics as those from the fluorescently labelled PAs, and how this differential wake could impact the physiology of the cell and the phenotypic variability between siblings. Interestingly, evidence in both bacteria and yeast suggests that functional refolding of aggregated proteins is favored considerably over degradation (Haslberger *et al.*, 2008; Wallace *et al.*, 2015) and could be a strategy to preserve cellular resources and allow improved resuscitation upon stress relief (Mogk *et al.*, 1999; Weibezahn *et al.*, 2004; Tessarz et al., 2008; Mogk et al., 2018). Together with the observation that natural PAs seem to consist of a complex and stress-dependent mixture of proteins and associated chaperones (Wallace *et al.*, 2015; Weids *et al.*, 2016; Govers *et al.*, 2018), this could imply that disaggregation, functional refolding and subsequent dilution of this complex array of proteins could reshape the proteomes of individual cells in a deterministic lineage-dependent fashion. However, further research is required to determine whether these potential proteomic differences would actually translate into observable and biologically relevant phenotypic variability.

In summary, PAs seem to be more than simple garbage bins. These molecular remnants from previous environmental insults can act as long-term, epigenetically inheritable memory elements that confer increased robustness to subsequent proteotoxic stresses. Moreover, concurrent PA segregation and disaggregation dynamics can add to lineage-dependent proteomic differences and drive differential phenotypic behavior. Although further study of these phenomena is required, it appears that ancestral torments can shape the behavior and heterogeneity of bacterial populations over several generations.

Fig. 1 Intracellular PAs as incentives for stress-borne heterogeneity. **a** Nucleoid enforced asymmetric segregation of PAs results in a PA-bearing and a PA-free subpopulation. PA-bearing cells are endowed with an increased cellular robustness against proteotoxic stresses. **b** Disaggregation and dilution of ancestral PAs results in a concentration gradient of disaggregated proteins among siblings, and thus in a lineage-dependent heterogeneity in individual proteomes. The numbers in each cell represent the degree of kinship to the PA-bearing cell (i.e. the number of generations a cell is removed from a PA-bearing cell).

Fig. 2 PA segregation and disaggregation dynamics leading to population heterogeneity. **a** Representative phase contrast, CFP fluorescence and overlay images of MG1655 Δ*lacY* pTrc99A-P_{trc}-*mCer-cI78*^{EP8} (left panel) and MG1655 Δ*lacY* Δ*dnaK* pTrc99A-P_{trc}-*mCer-cI78*^{EP8} (right panel) microcolonies grown for 2.5 hours after induction of the mCer-cI78^{EP8} PAs was halted (see Govers et al., 2018 for details on methodology). Scale bar corresponds to 5 µm. **b** Average cellular CFP fluorescence for all the MG1655 Δ*lacY* pTrc99A-P_{trc}-*mCer-cI78*^{EP8} cells (n = 178) of 30 microcolonies coming from 3 independent experiments. Cells were binned according to their degree of kinship to the PA-bearing cell. The more closely related a cell is to the PA-harboring cell, the more fluorescence it contains. P-value = 3.69×10^{-16} (linear mixed model with microcolonies considered as random factors). Error bars indicate the standard deviation.

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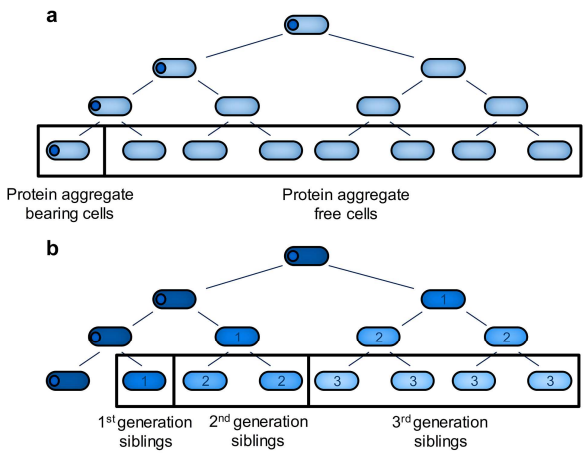

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347 Figure 1

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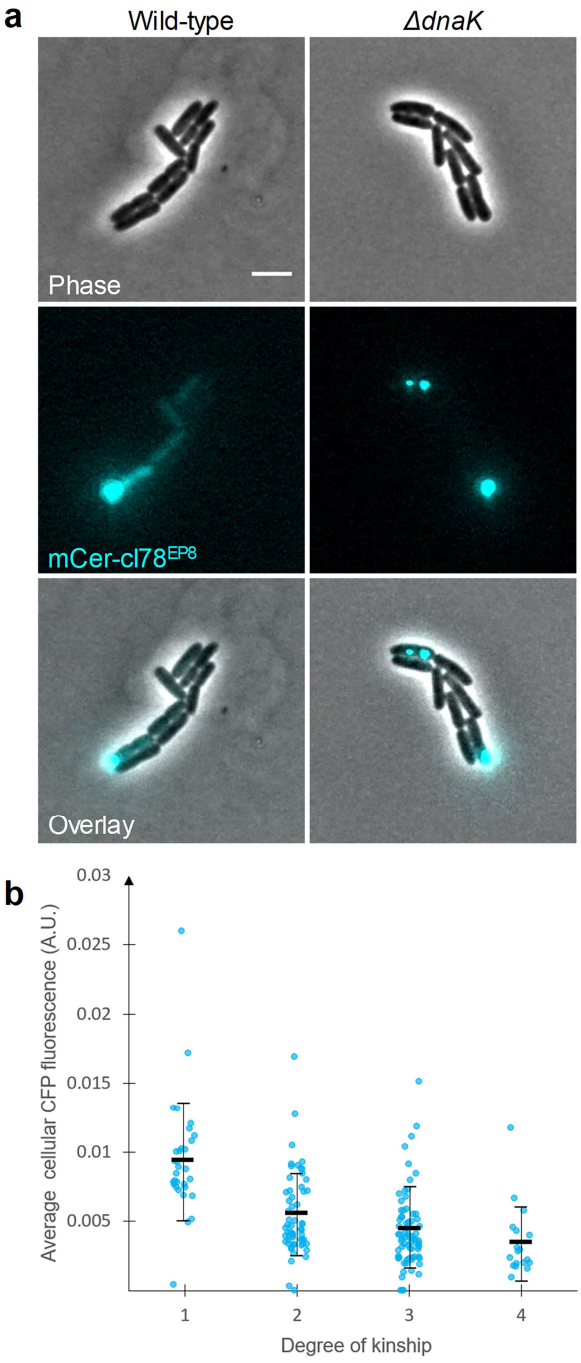

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350 Figure 2