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REVIEW ARTICLE

Role of Mitochondria in Generation of Phenotypic Heterogeneity in Yeast

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Abstract | A cell's phenotype is determined by its genome sequence and epigenetic state which translate into the biochemical reactions occurring inside the cells. As these biochemical processes are driven by small biological molecules, stochastic fluctuations may arise in the number of these biological molecules inside the cell and in the interactions between these molecules. These fluctuations can cause temporal variations in the cellular processes leading to variations in phenotype between two cells present in a population under identical environmental condition. Phenotypic variations in a population can enable a small fraction of cells to survive sudden changes in the environmental condition, as some of the cells are always prepared for such a change. Phenotypic variations can thus have very important implications for survival of a cell population and have been shown to affect our ability to treat human diseases—from eradication of a bacterial infection to treatment of cancer. In this review, I discuss the role of mitochondria, an important organelle in all eukaryotic cells, in generation of phenotypic heterogeneity. Mitochondria contains its own genome in multiple copies per cell and many proteins and RNA molecules required for proper functioning of mitochondria are present on the mitochondrial genome. Variations in number of copies of the mitochondrial genomes can thus lead to variations in mitochondrial functional state. As mitochondria have important roles in several cellular process, this can lead to variations in several cellular phenotypes including drug resistance. In this context, I also discuss the role of mitochondria in human diseases where mitochondrial heterogeneity could have important implications for disease progression and therapy. Thus, understanding the role of mitochondria in generation of phenotypic variation assumes significant importance in the context of human diseases as well as emergence of drug resistance.

1 Phenotypic Heterogeneity

Predictive **genotype–phenotype** mapping is one of the fundamental challenges in Biology[1–4]. The genetic material (genotype) of a cell contains information that manifest as the cellular phenotypes which range from gene expression patterns, topology of cellular networks, cell volume, cell surface properties to cell's growth rate and its response to environmental stimuli and drugs. However, we understand the principles of genotype–phenotype mapping only partially and there are several complexities that affect this mapping[4–10]. A comprehensive understanding of the mapping principles will enable us to mathematically predict values of phenotypes from a genotype. For example, we will be able to predict

Genotype: The information encoded in the genes and the genome of an organism.

Phenotype: An observable trait of a cell or of an organism that can be measured and quantified.

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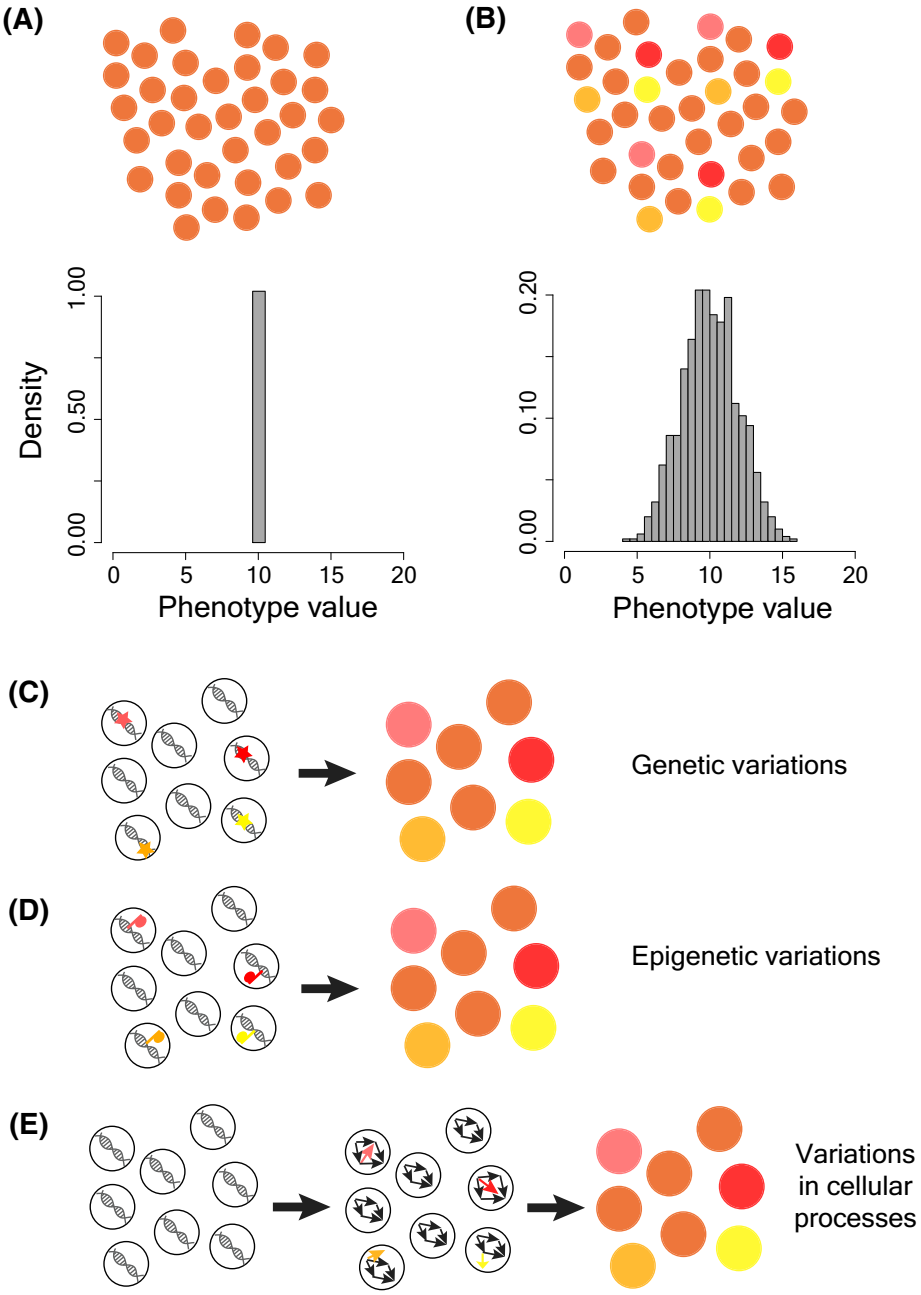

Figure 1: Phenotypic heterogeneity and its causes. **a** All cells in a phenotypically homogeneous population will show a single value for a phenotype. **b** Cells in a population exhibiting phenotypic heterogeneity will show different values for a phenotype thereby generating a distribution of phenotype values. **c–e** Phenotypic heterogeneity in a population can arise due to (**c**) presence of genetic variations in the population (**d**) epigenetic variations (**e**) stochastic variations in cellular processes. Each circle represents a cell and the colour of the circle represent the phenotype of the cell. Cells showing the same phenotype are represented by the same colour.

the disease risks of an individual from the genome sequence alone[11–14] and be able to determine antimicrobial resistance profile from the genetic composition of a bacterial population[15,16]. Such a predictive framework would enormously benefit healthcare, medicine, agriculture, and biotechnology industry[11–21].

A population of cells, be it microbial, fungal or mammalian, often exhibit a range of values for one phenotype in identical environmental condition—a phenomenon referred to as phenotypic heterogeneity (Fig. 1a, b). Phenotypic heterogeneity could arise in a population because of presence of genetic variations (Fig. 1c). Natural

microbial populations often harbor high genetic diversity[22–24]. Similarly, cancer cells have also been shown to be genetically extremely heterogeneous even within one tumor[25–27]. Alternatively, phenotypic heterogeneity can also arise due to variations in **epigenetic modifications** of the genome[28–30] (Fig. 1d). These modifications range from DNA methylations in prokaryotic cells to histone modifications and three-dimensional configuration of the genome in the eukaryotic cells[28–34]. Recent studies are beginning to show significant variations in the epigenetic modification patterns among individual cells in a population of eukaryotic and cancer cells[35–37] and these variations can drive phenotypic heterogeneity in a population.

Most interestingly, a cell population with identical genotype and epigenetic modifications can also exhibit a diverse range of phenotype values in identical environmental condition[38–42]. Such variations arise due to stochastic fluctuations in the cellular processes[43–45]. The protein and RNA molecules that carry out all the functions inside a cell are microscopic and thus, are subjected to fluctuations such as Brownian motion[46–49]. This can often lead to variability in initiation and termination time of cellular processes that can ultimately influence the cellular phenotypes. Alternatively, the biological molecules often interact with each other for executing cellular functions that eventually decide cellular phenotypes and small variations in these interactions from one cell to another can also lead to phenotypic heterogeneity. The heterogeneity arising out of variations in cellular processes are usually transient in nature and the cells switch from one phenotype to another phenotype concomitant with changes in the underlying cellular processes[50–52]. Thus, this kind of heterogeneity is often referred to as phenotypic plasticity where a cell can show different phenotypes in one environmental condition even though the genotype is the same[40, 51, 53–55]. In contrast to phenotypic heterogeneity generated due to genetic and epigenetic variations, phenotypes generated by variations in cellular processes are poorly inherited from one generation of cells to its progeny[56–59].

A prominent example of phenotypic heterogeneity arising out of variations in cellular processes is the phenomenon of gene expression **noise** where the expression level of a gene can vary among cells of an isogenic population in an identical environmental condition[43, 60–62]. Gene expression noise is generated by stochastic fluctuations in the processes of transcription and translation and is driven by the promoter sequence of the gene[63, 64], the location of the gene in the chromosome[65] and the nucleosome occupancy in the genomic region where the gene is present[64, 65].

2 Impact of Phenotypic Heterogeneity in Biological Systems

Phenotypic heterogeneity leads to a scenario where a small sub-population of cells differ in their phenotype from that of the majority of the population even in the same environment[22–37]. These phenotypes might be sub-optimal for survival and growth of the cells in that particular environment and can drag the population fitness down[66, 67]. However, such phenotypes could be advantageous when the cells are exposed to a new environment, such as occurrence of environmental stress or presence of growth inhibiting molecules in the environment[50, 51, 54, 68] (Fig. 2). Indeed, it has been shown that the genetic diversity in a microbial population can help some of the cells to survive in different antibiotics and can help in emergence of a drug-resistant population[69–72]. Similarly, presence of high genetic diversity and thereby high phenotypic heterogeneity in cancer cells complicates cancer chemotherapy, lead to evolution of drug-resistant tumor cells and cause cancer relapse[73–77]. Similarly, phenotypic variations generated due to variations in epigenetic modifications has been linked to drug resistance in cancer[77–80].

Phenotypic heterogeneity arising out of variations in cellular processes are reversible and allow a cell population flexibility to tune their phenotypes according to the environmental condition while keeping the genotype constant[68–70, 72, 81, 82]. Such phenotypic heterogeneity has been shown to have important roles in cellular decision making, in **bacterial spore** formation, in antibiotic persistence in bacteria and in **immune evasion** and drug resistance in cancer cells[55, 73, 76, 81–92]. One well-studied example of phenotypic heterogeneity generated due to stochastic variations in cellular processes is the phenomenon of persister cells[93, 94]. Persisters are a tiny sub-population of cells in a microbial population that can tolerate and survive even prolonged exposure to very high concentration of antibiotics. These cells can then regenerate the original cell population when the environmental stress or drug treatment is withdrawn[93–95]. Similarly, intra-tumor phenotypic heterogeneity arising from variations in cellular processes has also been observed in cancer and are thought to have important role for cancer metastasis and drug resistance[89, 90, 92].

Epigenetic modifications: Chemical modifications on the DNA that do not involve any change in DNA sequence.

Bacterial spore: A spore is a resistant dormant structure in bacteria formed in adverse environmental condition and can regenerate bacteria in favorable condition.

Immune evasion: Strategies usually employed by tumor cells and infectious organisms to avoid detection and killing by immune cells.

Noise: Variation in expression level of the same gene observed among individual cells in a population.

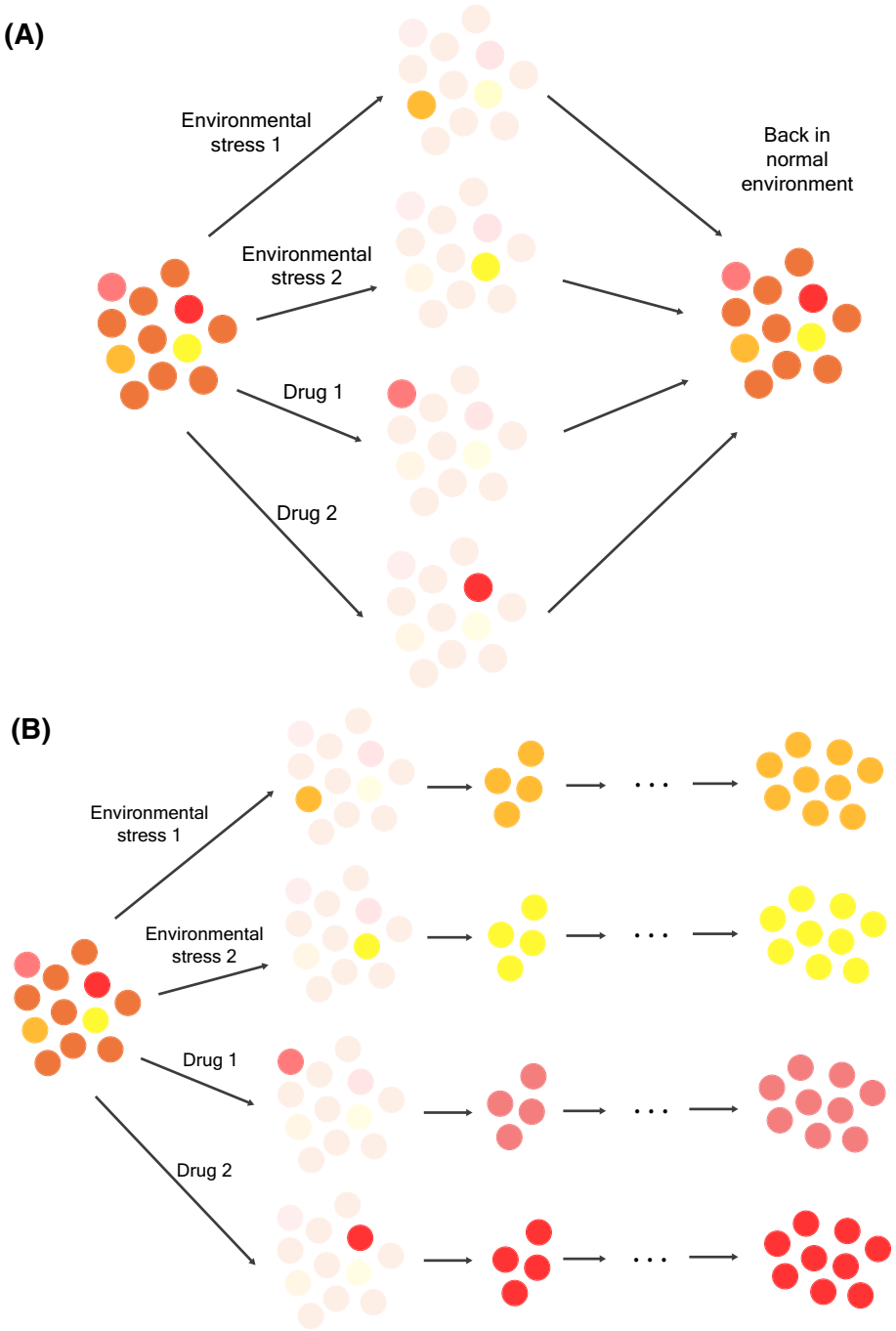

Figure 2: Impact of phenotypic heterogeneity in a cell population. **a** Phenotypic heterogeneity can enable a cell population to survive in diverse environmental stresses and drug treatments, as some cells showing phenotypic variations are prepared to survive these environmental changes. Once the environmental stress or the drug treatment disappears, these cells can regenerate the cell population. **b** A population with phenotypic heterogeneity can survive a permanent environmental change and can enable emergence of a resistant population in the long term.

3 Growth Heterogeneity and its Importance

Heterogeneity in growth rate is a common phenotypic heterogeneity observed in a cell population[68, 96–100]. Not all cells in a population consisting of genetically identical individuals (isogenic) grow at the same rate and variations in cellular processes can give rise to growth rate heterogeneity in a population. One of the most prominent examples of growth heterogeneity is observed in the case of persister cells in microbial populations[94, 95]. These cells are usually slow-growing and can tolerate high concentrations of antibiotic[94, 95, 101]. The persister cells have been shown to be generated by expression of toxin-antitoxin systems, where the toxin protein slows down growth[101, 102]. Such toxin-antitoxin systems are found in many human pathogens, suggesting a critical role of these pathways and thus, growth heterogeneity in persistent bacterial infections[103–106]. Similar growth heterogeneity has also been observed in cancer and has been shown to be important for tumor growth[99, 100]. However, the exact molecular causes of growth heterogeneity in eukaryotic and cancer cell populations are largely unknown.

Why are slow growing cells usually more resistant to antibiotics and drugs? The answer lies in the fact that most antibiotics and drugs inhibit the cellular pathways that are required for growth of cells. Since these pathways are already less active in slow-growing cells, the antibiotic and drug molecules become less effective. As a consequence, these cells can survive drug treatment regimens and in the long run, can lead to emergence of genetically stable drug resistant cell populations[106]. Thus, growth heterogeneity assumes significant importance with respect to the crisis of antibiotic and drug resistance[107–111].

4 Quantifying Growth Heterogeneity

A cellular phenotype is often measured for a population of cells which gives us a single value for the phenotype. In reality, this value represents the average of the **phenotype score** of all the individuals in the population (Fig. 1a, b). Quantification of phenotypic heterogeneity necessitates measurement of phenotype score for all individuals in a population. For example, heterogeneity in gene expression is measured by quantifying expression of a gene in all cells of a population, usually through flow cytometry[61, 63]. Similarly, for profiling growth heterogeneity of a cell population, one needs to quantify growth rate of individual cells in a population. This has been done in *E. coli* employing microfluidic devices[94], however with low throughput where the growth rate of only a few cells could be measured. High-throughput single cell growth rate quantification has been achieved in yeast where the growth rates of individual cells are measured through live-imaging microscopy[68, 112, 113]. This method has also been adapted to work in a high-throughput manner so that growth rates of thousands of cells can be measured in one experiment[113]. Briefly, single yeast cells are immobilized on microscopy plates and are grown over a period of time under appropriate growth conditions. Daughter cells are produced as cell division progresses and these cells tend to stay at the same location close to the mother cells and form microcolonies (Fig. 3a). Images of the microcolonies are acquired at regular intervals over a certain period of time. Computational processing of the images generates a relationship between the **microcolony** area (which correlates well with cell number) and time, which is then used to calculate the growth rate of the microcolony which started from a single cell (Fig. 3b). Once this data are collected for a large number of colonies, a growth rate distribution can be calculated (Fig. 3c). Work of several groups have shown that the growth distribution of the common laboratory strain of yeast shows deviation from a normal distribution and the population contains a small fraction of slow-growing cells[68, 112, 113]. This feature has been missed until now where only mean population growth rates have been measured. Thus, it is critical for us to develop techniques for quantifying single-cell phenotype as it can reveal biological systems in an unprecedented detail. This is helpful for a better understanding of the biological systems and can influence all areas of biological sciences and medicine. Single-cell-omics technologies (genomics, epigenomics, transcriptomics, metabolomics, proteomics) are steps in that direction[114–118].

Microcolony: An ensemble of small number of genetically related cells that can be observed under microscope.

5 Mitochondria as a Driver of Growth Heterogeneity

Although the phenotypic consequences of growth heterogeneity are widely known, the molecular mechanisms driving such variations are less known. Studies in bacterial persisters have shown a role of toxin-antitoxin systems in generating growth heterogeneity through activation of stringent response[119]. Similarly, variation in expression of virulence genes in Salmonella has been shown to lead to growth heterogeneity and ultimately, to antibiotic tolerance in a subpopulation

Phenotype score: The quantitative value of a phenotype determined experimentally.

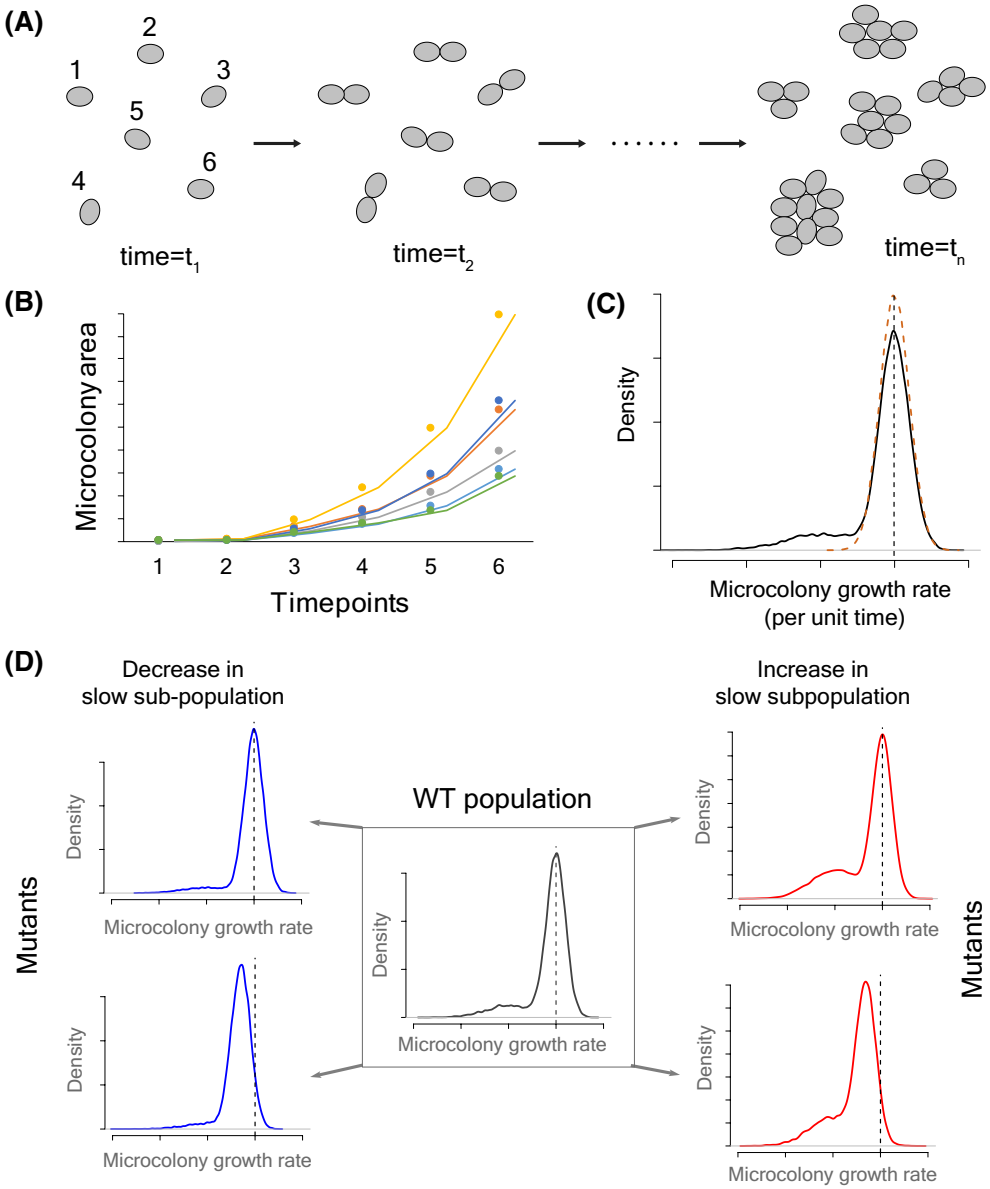

Figure 3: Measuring growth heterogeneity in a yeast population. **a** Single cells of yeast are immobilized and grown in appropriate growth condition. **b** Cells are imaged at regular time intervals; images are processed and the changes in area of the microcolonies over time are quantified for calculation of growth rate. **c** Measurement of growth rate across thousands of cells give rise to a growth rate distribution. The dashed lines show the mode growth rate and the red dotted curve shows a normal distribution with same mode growth rate. **d** Expected growth rate distribution of gene deletion mutants that reduce the fraction of slow-growers from the population (in blue) and growth rate distribution of gene deletions mutants that increase the fraction of slow-growers, with and without the change in mode growth rate.

of cells[120]. A recent study has shown a key role of stress-response **regulator** *RpoS* in growth rate modulation which enabled stress survival in bacterial populations[121]. Studies in yeast have identified the impact of genetic and non-genetic factors on growth heterogeneity across a wide range of yeast strains[112]. Among the non-genetic factors, variations in the level of cyclic AMP in cells have been shown to trigger growth heterogeneity and stress survival through stress responsive transcription factors *MSN2* and *MSN4*[122].

To systematically identify the cellular processes that contribute to the formation of slow-growing cells and thereby, generate growth

Regulator: A protein or a molecule that control and regulate a cellular process.

heterogeneity, one needs to carefully dissect the role of each gene present in the genome of the cell. Usually this is achieved through a genome-wide screen, by deleting one gene at a time and measuring the changes in the desired phenotype of the cell after a gene has been deleted. Any change in the phenotype score suggests a role for the gene that has been deleted, in generation of that phenotype. The same strategy was adopted for identifying the genes that contribute to growth heterogeneity in yeast *Saccharomyces cerevisiae*[113]. Approximately 1600 genes were deleted one by one and their effects on growth heterogeneity were identified. Wide variety of growth distributions were observed in the deletion strains. However, two classes of gene deletions were of interest as they led to identification of cellular processes causing slow-growth. Deletion of genes that lead to a reduction in slow-growing sub-population, with or without change in mode growth rate, are likely to be actively involved in generation of slow-growers. On the other hand, deletion of genes that lead to an increase in slow-growing sub-population are likely to buffer or regulate formation of slow-growing cells. A **gene functional enrichment analysis** showed that a majority of the genes whose deletion lead to increase in slow-growing sub-population were involved in mitochondrial function, suggesting a central role of mitochondria in generating growth heterogeneity[113].

Mitochondria is an important organelle present in all eukaryotic cells and are critical for energy generation in the form of ATP[123, 124]. The energy generation process requires five protein-complexes that are present in the inner mitochondrial membrane[125–128] (Fig. 3a). The complex I to complex IV are involved in pumping out proton from mitochondrial matrix to the intermembrane space (between inner and outer mitochondrial membrane). This leads to formation of a potential difference across the intermembrane space which is referred to as the mitochondrial membrane potential (MMP)[124, 125]. The complex V (or the ATP synthase complex) on the inner mitochondrial membrane harness this potential difference to generate ATP from ADP, in the process pumping protons into the mitochondrial matrix[127, 128].

Proper functioning of mitochondria requires approximately 800 proteins in the yeast *S. cerevisiae* and approximately 1300 proteins in human[129, 130]. Most of these proteins are encoded by genes present on the nuclear **genome** both in the case of yeast and human. In addition, mitochondria also contain its own genome and a small number of genes are present on the mitochondrial genome[131, 132]. Mutations in the genes important for mitochondrial function, whether the gene is present in the nuclear genome or in the mitochondrial genome, can affect normal mitochondrial function which can perturb several important cellular processes and thereby can affect the cellular phenotype[133, 134]. In humans, many such mutations in the nuclear genome and mitochondrial genome have been mapped and linked to a variety of mitochondrial diseases[135–137].

Unlike nuclear genome, a cell can harbor multiple copies of the mitochondrial genome. This can vary between 25 and 100 copies per cell in yeast[138] and between 100 and 100,000 copies per cell in human depending on the tissue where the cell is located[139–142]. Mitochondrial genome harbors genes encoding for some of the essential protein subunits of the complex IV and ATP synthase complex (complex V), as well as genes encoding mitochondrial rRNA and tRNA molecules. Interestingly, a cell can harbor mitochondrial genome with mutations along with non-mutated genomes, a condition known as heteroplasmy[143, 144]. Only when the number of mutated mitochondrial genomes exceeds the number of unmutated genomes, the effects of the mutations begin to manifest in the cellular phenotypes. However, the exact ratio between mutated and unmutated mitochondrial genomes beyond which these effects manifest is not known.

In addition, changes in mitochondrial genome copy number can lead to non-optimal expression level of mitochondrial protein and RNA molecules and can thereby affect normal mitochondrial function. Indeed, quantitative measurements of the mitochondrial genome (hereon termed mtGenome) copy number across sub-population of yeast cells suggested that mtGenome can spontaneously and substantially vary in copy number among members of a cell population who share the same nuclear genome[113] (Fig. 4b). Variation in mtGenome copy number altered mitochondrial membrane potential and perturbed normal mitochondrial function and ultimately, generated sub-populations of cells with different levels of mitochondrial activity[113] (Fig. 4b). Mitochondrial **DNA polymerase** (MIP1) in yeast has a key role in the maintenance of mtGenome copy number. Overexpression of MIP1 gene in yeast increased mtGenome copy number and reduced growth heterogeneity[113].

Variations in mtGenome copy number lead to generation of variable mitochondrial functional states that are likely to affect several

Gene functional enrichment analysis: Analyzing a list of genes to test whether genes associated with a specific function are enriched in the list than that would be expected by chance alone. This might suggest an important role of a functional class in a cellular process.

DNA polymerase: An enzyme that is responsible for replication of DNA molecules.

Genome: The genetic material of an organism and is present inside each cell of the organism. It contains all the gene sequences and non-coding DNA sequences.

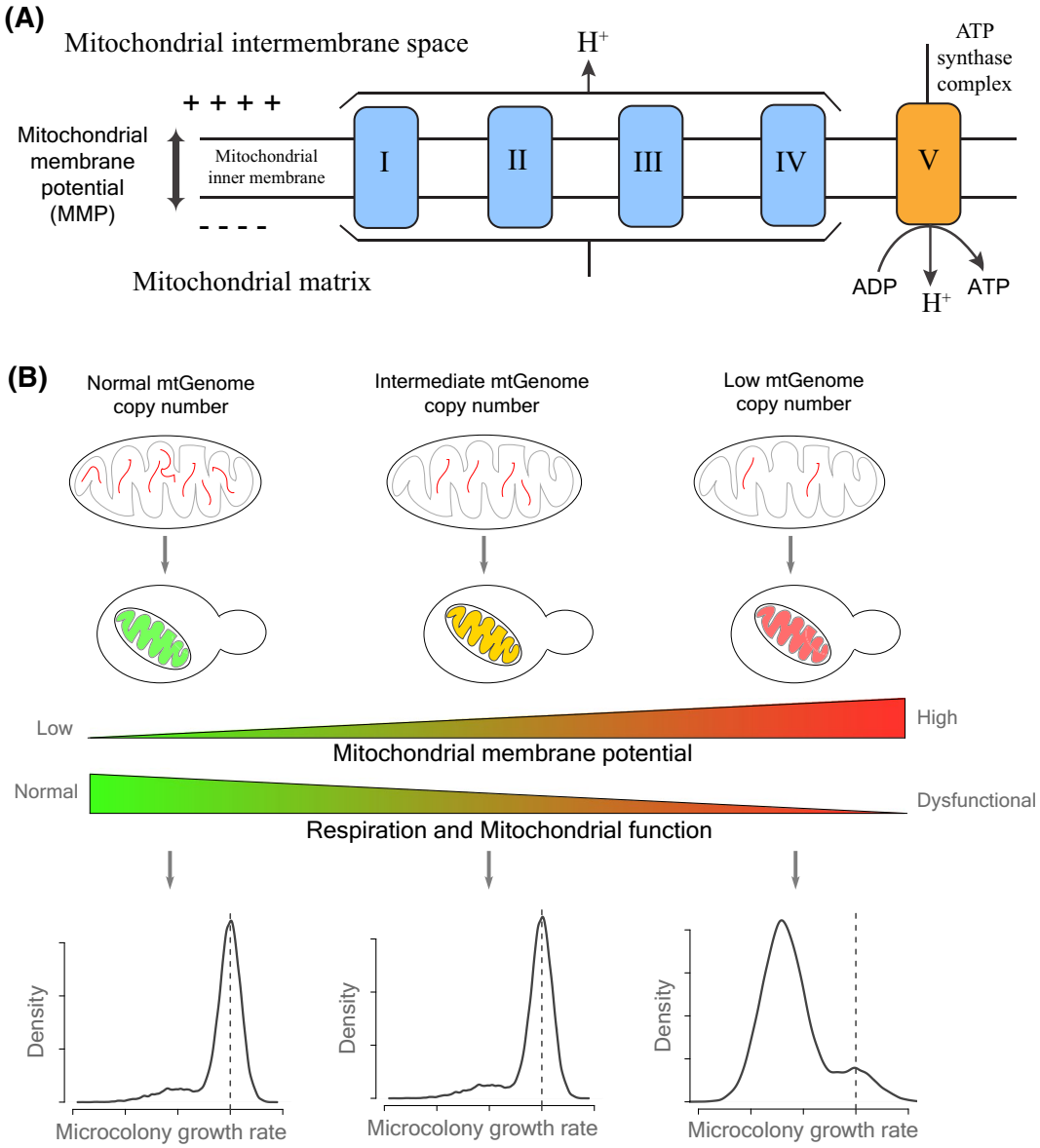

Figure 4: Variations in mitochondrial functional state is generated by variations in mitochondrial genome copy number. **a** Mitochondrial complexes I to IV are involved in exporting protons from mitochondrial matrix to mitochondrial intermembrane space. This leads to a potential difference across mitochondrial inner membrane that is known as mitochondrial membrane potential (MMP). **b** Variations in mitochondrial genome copy number in yeast cells lead to variation in mitochondrial membrane potential and mitochondrial function. Cells with mitochondrial dysfunction show enrichment for slow-growers.

key cellular processes and eventually alter cellular phenotypes. Specifically, cells with normal mtGenome copy number had normal mitochondrial function and were growing normally (Fig. 4b). At the other end of the spectrum, there were cells with very few copies of the mtGenome and had dysfunctional mitochondria and these cells were enriched for slow-growers. In between the extremes, there were cells with intermediate mtGenome copy number which were still able to grow normally (Fig. 4b). As mitochondria is essential for respiration, mitochondrial dysfunction will lead to less efficient oxidative phosphorylation, which, in turn, will reduce the ATP generation in the cell as well as affect other cellular processes[145,

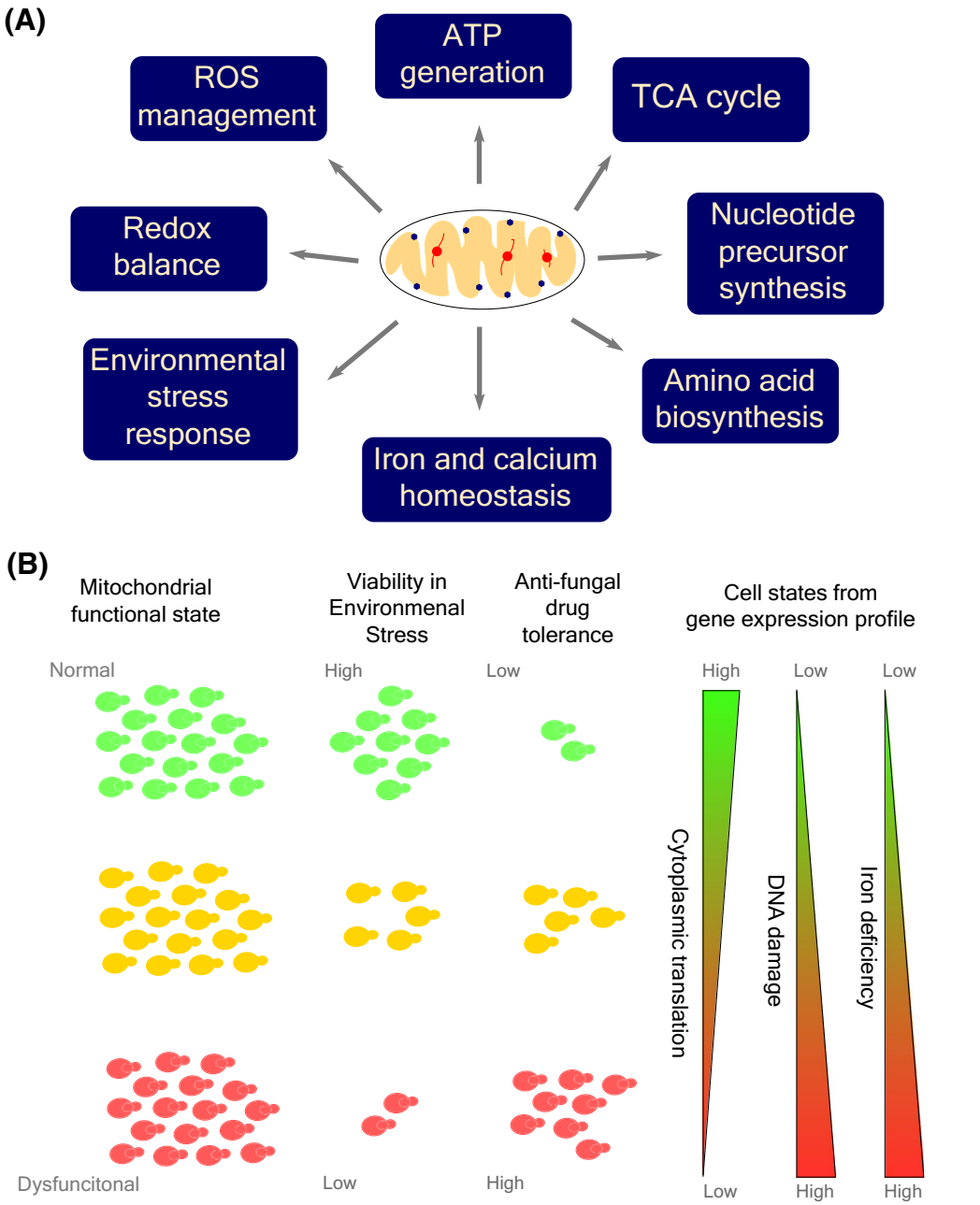

Figure 5: Variations in mitochondrial functional state drives variation in diverse cellular processes. **a** Mitochondria has important role in diverse cellular processes. **b** Changes in mitochondrial functional state lead to changes in cytoplasmic translation, iron homeostasis and influence survival of cells in the face of environmental stress and drugs.

[146]. This is likely to slow down growth of the cells. Thus, variations in mtGenome copy number led to variations in the mitochondrial activity, which ultimately caused growth heterogeneity in the yeast population.

6 Phenotypic Consequences of Variations in Mitochondrial Functional State

Besides ATP generation, mitochondria also play very important roles in diverse cellular processes (Fig. 5a). The tri-carboxylic acid (TCA) cycle that generates important intermediate metabolites for diverse cellular functions operates inside

mitochondria. In addition, the MMP is critical for homeostasis of iron and calcium inside the cell[145, 146]. The mitochondria are the hub for biosynthesis of heme, **iron-sulfur clusters**, amino acids and precursors required for nucleotide biosynthesis[146]. Further, during the process of oxidative phosphorylation and generation of ATP, reactive oxygen species (ROS) are generated which are then neutralized by mitochondrial enzymes[146–148]. The mitochondria also maintain a redox balance across the cells in the form of NADH/NAD^+ balance through aspartate-malate and malate-citrate shuttles that operate across mitochondrial membrane[146, 149, 150].

Given the important role of mitochondria in a cell, any change in mitochondrial membrane potential and mitochondrial functional state will lead to changes in diverse cellular processes. Changes in mitochondrial activity will primarily lead to changes in oxidative phosphorylation and ATP generation. Cells with mitochondrial dysfunction showed reduced expression of genes involved in oxidative phosphorylation and cellular respiration (Fig. 4b). Further, cells with mitochondrial dysfunction were growing slowly and thus, required less proteins for biomass formation, which was reflected in sharp decrease in expression of genes involved in cytoplasmic translation (Fig. 5b). Changes in mitochondrial potential will lead to changes in iron homeostasis and indeed, the cells with mitochondrial dysfunction displayed signs of iron starvation (Fig. 5b)[113, 151, 152]. Iron starvation can lead to reduction in iron-sulfur cluster biosynthesis which has been shown to cause genome instability[152]. Thus, not surprisingly, slow-growing cells showed signs of DNA damage as the DNA repair proteins were upregulated[113]. Interestingly, signs of DNA damage have been observed in slow-growing cells across several studies[96, 153]. Variation in mitochondrial content has earlier been shown to affect transcriptional rate and to generate gene expression noise in yeast[154, 155]. Similarly, analysis of transcriptome of cells with different levels of mitochondrial activity showed that variation in mitochondrial activity can also lead to heterogeneity in gene expression[113] (Fig. 6). Heterogeneity generated in gene expression from variations in mitochondrial content and functional state can thus drive phenotypic heterogeneity.

Mitochondria are involved in management of ROS damage in the cell and thus, have a critical role in cellular response to environmental stress. Thus, cells with mitochondrial dysfunction showed poor stress survival against salt and oxidative stress[113] (Fig. 5b). These cells also had lower expression of stress responsive genes[113]. In contrast, some of the other studies have shown that more stress-resistant cells tend to be slow-growing[68, 96]. However, it is likely that these stress-resistant cells form only a small fraction of the sub-population of slow-growers.

Cells have evolved mechanisms to sense changes in mitochondrial functional state and to remodel their metabolism accordingly. Specifically, mitochondrial dysfunction activates **retrograde signaling** pathway which remodels nuclear gene expression through activation factors RTG1, RTG2 and RTG3 in yeast[156, 157]. Since the normal **TCA cycle** is disrupted in the cells with dysfunctional mitochondria, retrograde signaling enables these cells to produce necessary metabolites bypassing the TCA cycle[151, 152]. Very interestingly, mitochondrial dysfunction also activates the **multidrug efflux pump** PDR5 through the transcription factor PDR3, however, the complete cellular pathway for this activation is unknown[158, 159]. The drug efflux pumps help yeast cells survive and grow in antifungal drugs by actively pumping the drugs out of the cells. Thus, as expected, cells with mitochondrial dysfunction showed higher expression of the PDR5 gene and were able to survive and grow in antifungal drug whereas the cells with normal mitochondrial function were killed[113] (Fig. 5b). Taken together, changes in mitochondrial function led to variations in diverse cellular phenotypes including environmental stress tolerance and drug resistance.

7 Mitochondrial Heterogeneity and Human Diseases

As mitochondria are essential for diverse cellular functions, disruptions in normal mitochondrial function can perturb cellular homeostasis and can lead to cellular abnormality. Such abnormality can manifest themselves as disease in humans. Indeed, mitochondria has been linked to many diseases in humans. Mitochondrial diseases range from Alpers-Huttenlocher syndrome, Pearson syndrome, Mitochondrial Encephalopathy, Lactic acidosis, and Stroke like episodes (MELAS), Mitochondrial Neuro-gastrointestinal Encephalopathy Disease (MNGIE), Amyotrophic lateral sclerosis (ALS), to neurodegenerative disorders such as Alzheimer's and Parkinson's diseases to diabetes, cardiac diseases, and respiratory diseases[135–137, 160–165]. Mitochondria has also been shown to be a key component of cellular aging in yeast model[166, 167]. In addition, changes in mitochondrial genome copy number has also been observed in several cancers[168, 169], suggesting an

Iron-sulfur clusters: These are organometallic ensembles of iron and sulfur atoms and serve as cofactor for many important biological enzymes.

Retrograde signaling: Intracellular signaling from mitochondria to nucleus for modification of cellular processes in accordance to the internal state and external environment of the cell.

TCA cycle: A central part of the cellular metabolism that generates several important cellular molecules required for growth and survival of cells.

Multidrug efflux pump: Protein complexes present on cell surface that actively recognize drug molecules and help transport them out of the cell. These pumps are a major cause of failure of drug treatments.

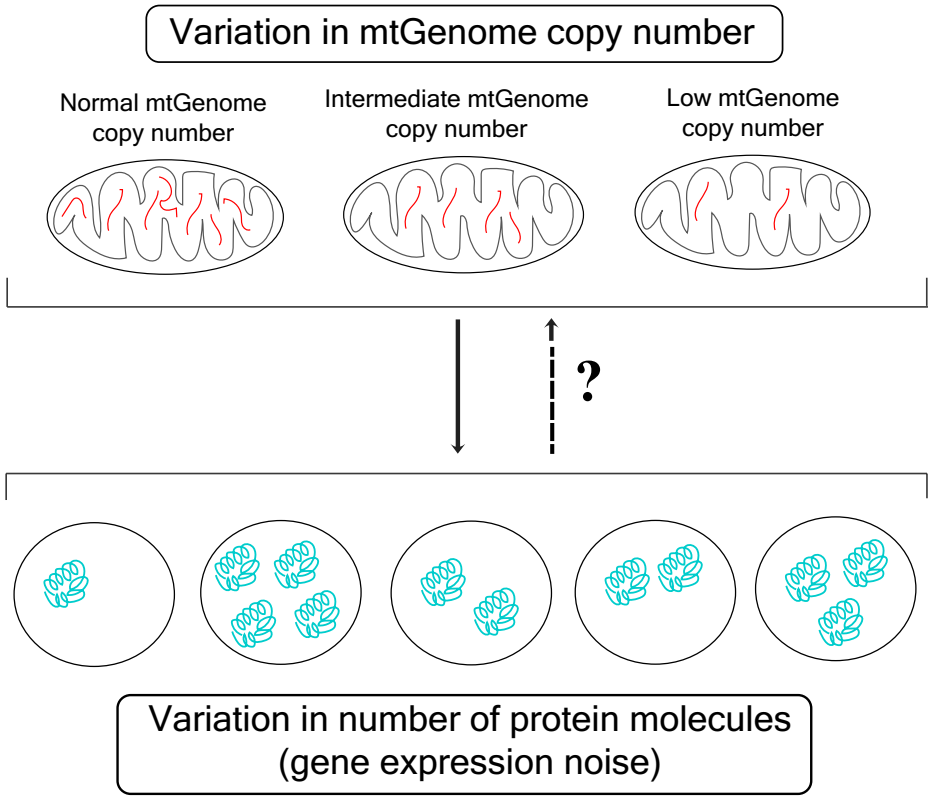

Figure 6: Variations in mitochondrial genome copy number lead to mitochondrial activity which lead to variations in gene expression. The question how the variations in mtGenome copy number arise and whether gene expression noise has any role in this process, however remains to be answered.

important role of mitochondria in cancer progression and malignancy. Although, it is widely presumed that the disruptions in normal mitochondrial function lead to problems in ATP generation, which, in turn, results in disease state, recent studies are painting a much more complex scenario. For example, problems in iron transport and iron homeostasis as a result of mitochondrial dysfunction has been shown to underlie cardiomyopathy and neurodegenerative disorders[164, 165].

Although many studies till date has identified mutations in the nuclear genome as well as in the mitochondrial genome across diverse mitochondrial diseases[135–137, 162], how mitochondrial heterogeneity impacts disease phenotype and drug resistance is less clear. Several studies are uncovering a role of heterogeneity in mitochondrial genome sequence, heteroplasmy, in human diseases[143, 170–172]. Interestingly, a very recent study has shown existence of three different energetic states with distinct mitochondrial structure and dynamics in ovarian tumor initiating cells[173], suggesting potentially important role of such heterogeneity in tumor development and progression[173, 174]. In addition, mitochondrial heterogeneity has been suggested to be important for pluripotent stem cell reprogramming and differentiation[174, 175].

8 Summary and Future Scope

Phenotypic heterogeneity can arise due to presence of genetic variations, due to epigenetic variations or due to stochastic variations in cellular processes from one cell to another. As a consequence, a cell population is always likely to exhibit a range of phenotypic scores whether the population contains cells with identical genotype or not. Although our understanding of phenotypic heterogeneity generated due to genetic and epigenetic variations is quite mature, we are only beginning to uncover the phenotypic heterogeneity generated due to variations in cellular processes. As the cellular processes involve small biological molecules, variations in cellular processes due to variability in interaction between molecules and variability in initiation and termination of cellular processes is inevitable. There are thousands of

biochemical reactions that occur in a cell at a given point of time and thus, there are thousands of ways in which two cells can differ in their cellular processes. Phenotypic heterogeneity generated due to process variations can thus complicate predictive genotype–phenotype mapping.

Phenotypic heterogeneity can have far reaching implications for our ability to treat human diseases. Small phenotypic variations among cells in a population can heavily influence the viability of cell populations. Phenotypic variations in microbial cell population can enable a small fraction of cells to survive environmental stress and antibiotic treatment, thus ensuring the survival of the microbial population. This process can lead to persistence of microbial infections even after antibiotic treatment and in the long-term, could aid in the emergence of antibiotic-resistant populations. Presence of phenotypic heterogeneity in a tumor cell population can affect efficacy of anti-cancer therapy where a small sub-fraction of cells can survive the treatment and can lead to relapse of cancer. This can also enable drug-resistant cancer cells to emerge and proliferate.

Variations in growth rate among individual cells in a population is a common phenotypic heterogeneity observed in microbial and tumor cell populations and have been shown to have important impact on their ability to resist and survive drug treatment. A systematic genetic screen in yeast has established that the growth heterogeneity is primarily driven by mitochondria. Variations in mitochondrial genome copy number lead to variations in mitochondrial membrane potential and eventually to variations in mitochondrial functional state. As mitochondria perform important roles in several key cellular processes, variations in mitochondrial functional state leads to variations in several cellular phenotypes. Most interestingly, mitochondrial dysfunction caused by reduction in mitochondrial genome copy number leads to higher drug resistance in yeast. As depletion in mitochondrial genome copy number has also been observed across different cancers, it would be interesting to investigate whether this depletion might also enable tumor cells to resist anti-cancer drug treatments.

Variation in mitochondrial genome copy number has been shown to underlie growth heterogeneity in yeast. Such variations have also been observed across many human diseases and cancer. However, we do not understand how such variations in mitochondrial genome copy number arise and what are the cellular processes that contribute to this variation. Mitochondria divide and merge through the process of fission and fusion[176, 177]. Mitochondrial genome is present in mitochondrial nucleoids which are inherited along with mitochondria from the mother cell to the daughter cell during cell division[178, 179]. Thus, it is conceivable that the partitioning of mitochondrial nucleoids during cell division is asymmetric [180, 181] and gives rise to variability in mitochondrial copy number. Alternatively, stochastic variation in expression level of cellular machinery responsible for maintenance of mitochondrial genome could also contribute to this process.

Changes in mitochondrial functional state has been observed across a plethora of human diseases. However, our understanding of how these changes and the disease phenotypes are connected to the disease mutations is quite limited. As mitochondria participate in several important cellular processes, it is conceivable that specific mutations and copy number changes of the mitochondrial genome can alter other cellular processes besides ATP generation. Further, it is also likely that these changes in mitochondrial genome can drive cell-to-cell heterogeneity and could have potentially important impacts on disease progression and outcomes. Thus, a concrete understanding of the effects of the mitochondrial genomic changes on cell-to-cell heterogeneity and disease phenotypes is the need of the hour. Spontaneous variations in mitochondrial genome copy number in yeast cells and presence of mutations and copy number variations in mitochondrial genome across many human diseases show that in eukaryotic cells with identical nuclear genome phenotypic heterogeneity can be driven by an organelle. It remains to be seen how cellular organelles other than mitochondria contribute to phenotypic heterogeneity.

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