OPINION

Origins of regulated cell-to-cell variability

Berend Snijder and Lucas Pelkmans

Abstract | Single-cell measurements and lineage-tracing experiments are revealing that phenotypic cell-to-cell variability is often the result of deterministic processes, despite the existence of intrinsic noise in molecular networks. In most cases, this determinism represents largely uncharacterized molecular regulatory mechanisms, which places the study of cell-to-cell variability in the realm of molecular cell biology. Further research in the field will be important to advance quantitative cell biology because it will provide new insights into the mechanisms by which cells coordinate their intracellular activities in the spatiotemporal context of the multicellular environment.

The question of how mammalian cells work in molecular, dynamic and morphological terms has typically been addressed with cell-population-averaged techniques and by focusing on individual cells. Given the variable behaviour of individual cells, however, both approaches probably fail to resolve the full spectrum of cellular activities present in cell populations[1–3]. Despite much progress in measuring cell-to-cell variability[4,5], few variable single-cell characteristics have been put into the context of possible predetermining factors, such as the cell's history and microenvironment. Technical advances now allow us to see when and where individual cells display their properties of interest, and to what extent these properties are predictable.

As biological systems are increasingly being analysed at the single-cell level in a large number of cells, various new deterministic influences (that is, influences on a prior state or activity of a cell that determine the subsequent activity or state of that cell) of cell-to-cell variability are being revealed. Our recent work on population context-dependent cell-to-cell variability in human tissue culture cells[6] shows that, at least for virus infection efficiency and endocytosis and the underlying regulatory systems of these processes, this variability is strongly regulated in response to factors that shape a population of cells, such as cellular crowding and cell–cell contacts. Moreover, recent studies have revealed the presence of predetermining factors in the cell-to-cell variability of apoptosis[7–9], cancer heterogeneity[10–13], stem-cell differentiation[14,15], the induction of pluripotency[16], and nuclear factor-κB (NF-κB) signalling[17], as well as in λ phage infection in *Escherichia coli*[18,19], the yeast pheromone response[20,21] and various other heterogeneous activities in prokaryotic and eukaryotic cells[22–26].

Based on this, we propose in this article that quantifying the full spectrum of activities and states that occur within a population of non-differentiating cells is essential for correctly understanding their underlying regulation. We first explain the semantics used in this field. This is followed by a short historical context of cell-to-cell variability and by discussion of recent work that has revealed various new deterministic sources of cell-to-cell variability. We then argue that both the discussion on, and our understanding of, cell-to-cell variability will be aided by quantifying the balance between deterministic and stochastic contributions, and by identifying the factors that determine the variable behaviour of cells. Such regulated cell-to-cell variability will be a goldmine for the future of molecular cell biology, both in methods, by harnessing it to reveal molecular regulatory networks (BOX 1), and in concepts, as it allows the study of cellular activities in the spatiotemporal context of a cell population. For recent reviews on the impact of stochastic contributions on cell-to-cell variability, we refer the reader elsewhere[27–31].

The semantics of noise

Cell-to-cell variability is often referred to as cellular noise because it may arise from the inherently probabilistic and discrete nature, called intrinsic noise, of intracellular biochemical reactions. Intrinsic noise can be experimentally observed inside single cells at the molecular level[32,33] (recently reviewed in REF. 34). Progress in the study of intrinsic noise over the past 10 years has shown that it has been adjusted during the functional evolution of signalling pathways. Intrinsic noise might be the reason why cells have developed certain network motifs to regulate cellular behaviour, as it allows them to modulate the level of uncertainty in the outcome of regulatory systems[35,36]. Such regulatory systems or decisions, in which the outcome of a cellular event is at least partially the result of intrinsic noise, are said to be stochastic. Numerous examples of stochastic regulatory systems have been reviewed[31,36].

Recently performed genome-wide studies of cell-to-cell variability in mRNA or protein levels in *E. coli* and *Saccharomyces cerevisiae* have indeed revealed a tremendous variation in the molecular make-up of genetically identical cells from the same population[37,38]. However, these and other studies have also indicated that the major source of variability in protein levels does not stem from intrinsic noise, but rather from upstream influences[37–40]. Such upstream sources of variability, often termed extrinsic noise, could in turn reflect either stochastic or deterministic influences. The existence of upstream stochastic influences does not, however, mean that the system under investigation is itself stochastic. And, although determinism does not strictly imply regulation, the presence of upstream deterministic influences is likely to reflect complex regulatory systems that control the cell's physiology.

To avoid confusion, it is important to distinguish between the terms cellular noise (both intrinsic and extrinsic) and cell-to-cell variability. Because the term cellular noise

Box 1 | **Cell-to-cell variability reveals circuit architecture**

Population context-dependent cell-to-cell variability is a mixed blessing. On the downside, this inherent variability of mammalian cells can significantly interfere with population-averaged measurements if population context parameters are not kept equal between experiments[6]. However, cell-to-cell variability can be harnessed to reveal novel links and causal interactions between phenotypic properties and molecular activities[6,88–90]. This relies on computational methods from machine learning and engineering, including Bayesian network inference and auto-correlative and correlative analyses, which infer relationships between individual parameters based on their correlation and mutual information using quantitative measurements of single cells. Quantitative single-cell measurements can be obtained by microscopy-based image analysis and fluorescence-activated cell sorting (FACS)-based assays, although spatial and temporal single-cell variability can only be revealed by microscopy. Moreover, FACS currently allows for a higher number of fluorescent reporters to be measured simultaneously[91], whereas the amount of cellular phenotypic and environmental properties obtained by microscopy is mainly limited by the available image analysis algorithms[92–94], although the repertoire of these algorithms is growing. Finally, the combination of laser capture micro-dissection and the development of ever more sensitive '-omics' tools will allow context-dependent and broad-scale molecular profiling of individual cells[95]. Another advantage of studying cell-to-cell variability comes from the increased sensitivity of the measurements. Indeed, the variation in individual cell shapes is larger within a single unperturbed cell population than between populations perturbed with a set of cytoskeleton-targeting drugs[67].

assumes that "random or irregular fluctuations or disturbances which are not part of a signal ... or which interfere with or obscure a signal"[41] are causing the observed cell-to-cell variability, we prefer to use it only when this randomness has been explicitly shown and unknown deterministic influences have been ruled out.

It is also important to make clear distinctions between single molecular readouts and readouts of cellular activity or phenotype, as it is difficult to link molecular cell-to-cell variability to phenotypic cell-to-cell variability without measuring both and testing causal interactions. For instance, the fact that mRNA levels do not, at a global level, correlate with their corresponding protein levels[37,42], and that protein levels may hold no information on protein subcellular localization[43,44] or post-transcriptional modification[45] — both of which regulate the activity of many proteins — complicates the extrapolation of noise in the transcription of single genes to variability in cellular activity.

There is a fundamental problem in empirically separating stochasticity from determinism at the level of cellular phenotypes and activities. We can only state that a certain fraction of observed cell-to-cell variability is deterministic (there may be more determinism than is currently known) and so, at most, only the remaining fraction of variability is stochastic[30]. As the regulation of most cellular activities is highly complex and still poorly understood, particularly in mammalian cells, we may expect seemingly stochastic variation in cellular phenotypes to become explained by newly discovered regulatory mechanisms. Indeed, various '-omics' approaches reveal the existence of large numbers of novel molecular interactions[46–48] and regulators of cellular activities[49,50]. Many of these may contribute to determining cell-to-cell variability. Furthermore, to make a system robust to intrinsic noise[51], a large number of regulatory mechanisms must be put in place[52], which might explain why cellular systems have evolved the complexity we observe. For instance, systematic overexpression of proteins in yeast[53] and the large-scale addition of new regulatory links in transcriptional networks in *E. coli*[54] have revealed remarkable robustness in cellular growth.

A history of cell-to-cell variability

The concept of cell-to-cell variability emerged in the pre-molecular biology era from studies on *E. coli*. Delbrück realized in 1940 that, inside living cells, uncertainty in the occurrence of chemical interactions may affect the outcome of cellular decisions[55], and he proposed that this might partly explain the large variability observed in the number of phage produced per virus-infected cell[56]. However, he also suggested that such variability might have predetermined influencing factors, such as cell size. In 1957, Novick and Weiner suggested that, in bacteria, the decision to switch from glucose to lactose metabolism at intermediate lactose levels could be made by random fluctuations in the levels of certain regulatory components involved[57]. Much later, Arkin and colleagues applied stochastic mathematical modelling of chemical interactions[58] to the regulatory circuit that decides between two possible outcomes of λ phage infection in bacteria: the lytic versus lysogenic switch[59,60]. The stochastic model predictions fitted with experimental population-averaged data, suggesting that this switch is stochastically determined[59]. However, Herskowitz and others in the 1970s suggested that this switch might be predisposed by the nutrient status in *E. coli* cells to maximize viral yield (reviewed in REF. 60), but the effect of this determining factor at the single-cell level remained elusive. In 1976, Spudich and Koshland studied non-genetic heterogeneity in the switching dynamics between two modes of migration in bacterial chemotaxis[61]. Using single-cell tracking and repeated stimulation of the same individual cells, they demonstrated strong individuality and persistent memory in switching rates. Uneven partitioning of molecular regulators during cell division, and not cell size or cell cycle effects, was suggested as the cause of this variability[61]. The view that stochasticity in biochemical interactions largely influences cell fate decisions gained strength when it was demonstrated that the expression of a gene inside a single living prokaryotic cell could show a significant amount of intrinsic noise[32,33].

However, pioneering work with mammalian tissue culture cells in the 1960s and 1970s showed large variability between individual cells in growth rate, cell migration and cell shaping as a consequence of cellular crowding and cell–cell contacts[62,63]. Furthermore, in 2005, the amount of stochastic noise in the yeast switch to a mating state in response to pheromones was shown to be surprisingly small[64]. Predetermined factors in the levels of regulatory components and the relevant signalling capacity of cells accounted for approximately 99% of the variability in single-cell responses[64]. Subsequent analyses of the underlying regulatory circuits identified auto-regulatory negative feedback in the localization of a mitogen-activated protein (MAP) kinase scaffolding protein, through which fast, robust and deterministic responses are achieved despite intrinsic noise[21,65].

New-found determinism in cell fates

Two classical examples of cell fate switches that were thought to be largely unpredictable or stochastic have recently been shown to contain more determinism. It was shown that lysis after λ phage infection occurs primarily in large *E. coli* cells, whereas lysogeny occurs in small cells[18,19]. Similarly, it was recently shown in *E. coli* that the lactose operon switch, which can create bistability in β-galactosidase expression within a population of cells, is not largely stochastic at the

single-cell level, but is strongly predetermined by the cell's physiology and growth rate[25]. In both examples, the quantification and correlation of the cellular outcome with phenotypic properties of cells were used to explain cell-to-cell variability, similar to our findings that population context parameters predict the efficiency of virus infection, endocytosis and membrane lipid composition in mammalian cells[6] (FIG. 1). In all cases, specific phenotypic properties of single cells were strong predictors of cell fate or cellular activity outcome. Therefore, to understand the sources of variability in the outcome of regulatory networks, it is of great importance to consider their embedding in the cellular physiology. This also applies to the use of synthetic biology in addressing such research questions (Supplementary information S1 (box)). Calling heterogeneous cellular activities 'noisy' a priori overlooks the interesting and often unknown sources of regulated cell-to-cell variability.

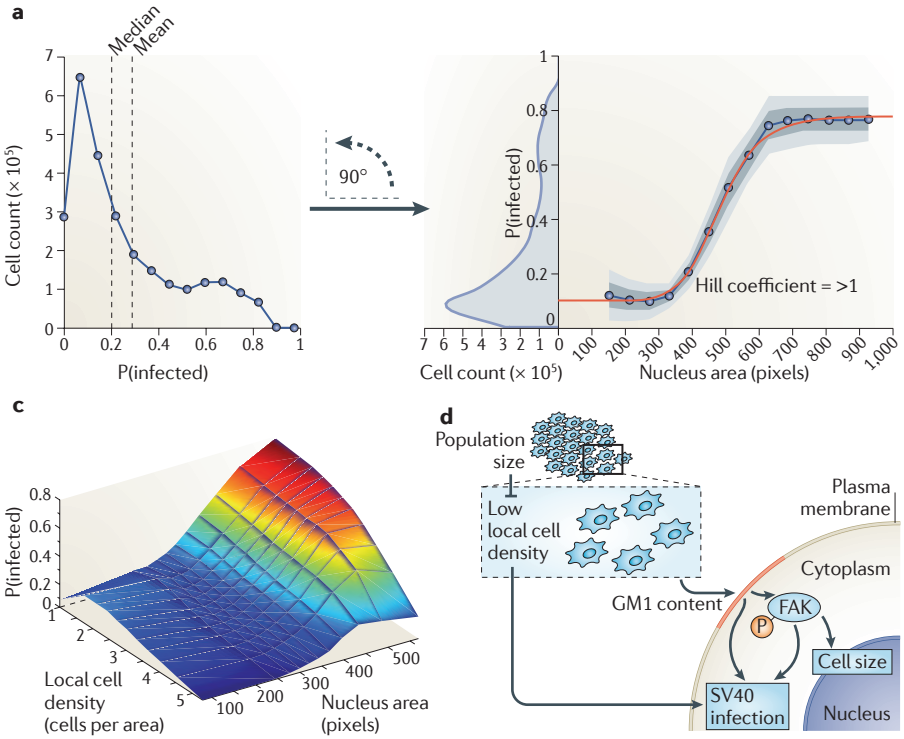


Figure 1 | **Explaining the regulated cell-to-cell variability of SV40 infection. a** | A histogram of single-cell simian virus 40 (SV40) infection probabilities (P(infected); *x*-axis), as measured in 2.6×10^6 HeLa cells. Neither median nor mean probabilities (indicated by dashed lines) are a good representative of the measured single-cell probabilities. The coefficient of variation (CoV), a common dimensionless noise measure calculated by dividing the mean by the standard deviation, is 0.78 for the probability of infection. **b** | The observed cell-to-cell variability can be largely 'explained away' by a single predictor, in this case cell size (nucleus area). Importantly, in this example, SV40 does not induce an increase in cell size. The local average probability of infection (*y*-axis, blue line and circles) is plotted against nucleus area (*x*-axis). Note that the *y*-axis in this graph is the *x*-axis of the graph in **a** and that the infection probability distribution of **a** is plotted as a function of nucleus area (cell size). Dark and light grey regions indicate, respectively, the ×0.5 and ×1 local standard deviations of infection probabilities. A fitted Hill function (red line, adjusted to different minima and maxima) reveals a Hill coefficient of >1, which indicates strong switch-like behaviour of SV40 infection probability in HeLa cells as a function of cell size. The average CoV is 0.31. **c** | When considering more predictors, the noise can be further reduced. The same single-cell SV40 probabilities of infection are now plotted as a function of both nucleus area and local cell density. The average CoV is further reduced to 0.21. The colour corresponds to the probability of infection. **d** | Bayesian network structure inference performed on single-cell data reveals part of the underlying molecular network that determines the regulated cell-to-cell variability observed in SV40 infection, including glycosphingolipid monosialotetrahexosylganglioside (GM1) levels at the plasma membrane and activated focal adhesion kinase (FAK). The displayed circuitry contains a coherent feedforward loop, which displays synergy to cancel out intrinsic noise while amplifying regulated cell-to-cell variability (see also BOX 2). Results adapted from REF. 6.

Cells put in population context

What, then, determines variations in the phenotypic properties of genetically identical single cells? Based on recent studies, we propose that these variations are largely determined by the inherent properties of growing cell populations that create a large spectrum of microenvironmental differences to which cells adapt[6,23], combined with the non-genetic memory of phenotypic states and protein levels[9,66,67]. As soon as a single cell starts to divide, whether it is a bacterium, a yeast cell or a mammalian cell, differences in cell–cell contacts and the available space will arise among the single cells, even in experimental settings where culture conditions are kept constant. That this is the predominant source of cell-to-cell variability is also suggested from the measurement of cells grown on micropatterns. When single human cells are forced to adopt an identical size and shape, the subcellular distribution of intracellular organelles is remarkably constant[68], illustrating that this system has little intrinsic noise.

The population context of single-cell organisms. In bacteria, there is ample evidence that cells in colonies display complex patterns of multicellular behaviour, which improve the overall fitness of the population[22,31,69,70]. In order to accomplish this, bacteria communicate with other cells, form cell–cell contacts and sense the local cell density through secreted signalling molecules in a process called quorum sensing[71,72]. This allows cells to adapt a multitude of their activities to how crowded the population is.

As a well-studied example, *Bacillus subtilis* can undergo several mutually exclusive switch-like cell-fate decisions, which are regulated by the integrated sensing of environmental cues, including cell density and nutrient level[23,73,74]. Although these different cell fates were proposed to be determined by random switching mechanisms resulting from intrinsic noise[75,76], recent studies revealed the presence of several predetermining factors in these *B. subtilis* cell-fate switches, including the growth speed of individual cells and as-yet-unidentified factors[23,24,77]. The different cellular behaviours determined by the population context include sporulation, genetic competence and motility, with sporulation and genetic competence occurring at high local cell densities, and motility occurring in basolateral regions of the population (reviewed in REF. 78).

Mammalian cell population context. In growing adherent mammalian cells, a few cell divisions combined with cell motility will create a wide variation in local cell densities, cell–cell contacts, relative location, and the

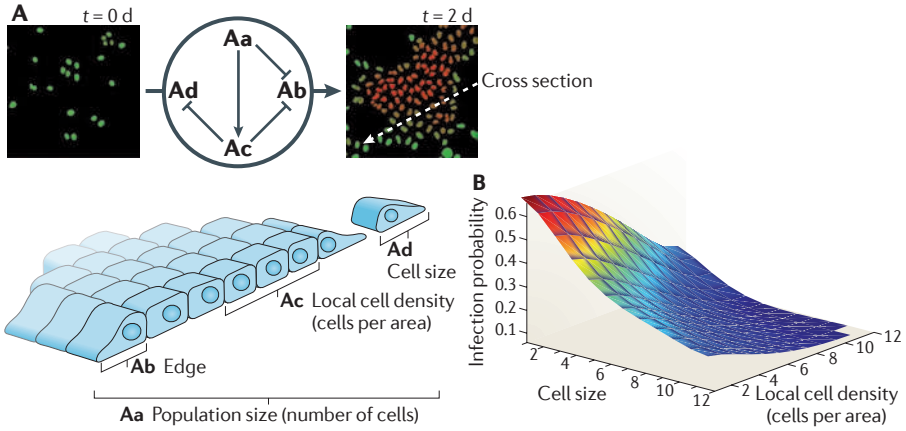


Figure 2 | **Measuring cellular activities in the full spectrum of the mammalian population context. A** | Two snapshots of a movie, recorded with time-lapse fluorescence microscopy, of a population of human cells growing for 2 days in culture. *t* = 0 d shows single cells at the start of the movie and *t* = 2 d shows single cells after 2 days of continuous growth. Computer-segmented nuclei, pseudo-coloured from green to red with increasing local cell density, are shown. As the cell population (**Aa**) increases, the number of cells at the edge (**Ab**), bordering empty space, decreases and the local cell density (**Ac**) increases. Owing to the increased local cell density, the cell size (**Ad**) decreases. These causal interactions that shape a population of cells are indicated in the schematic network linking the two snapshots. The result of cellular growth is a population of cells with widely varying population contexts and phenotypic states. **B** | Because many cellular activities are regulated by population-context dependent parameters, any cellular activity should be measured across the full spectrum of phenotypic states present in a population of cells. As a specific example, we show virus infection in human cells across the spectrum of local cell density and cell size. In this hypothetical case, the virus preferentially infects small cells growing in sparsely populated areas. Such a population-context dependent decomposition of cell-to-cell variability is essential for a full understanding of the activity[6]. The colour corresponds to the probability of infection.

amount of free space per cell[6]. Combined, these parameters constitute the population context of an individual cell, to which each cell adapts its physiology. The fact that molecular mechanisms that sense these parameters are present in bacterial, yeast and mammalian cells underpins the fundamental and ubiquitous importance of such population-dependent behaviour. These adaptations might be in gene transcription, protein translation, cellular growth, rate of proliferation, sensitivity to apoptosis, metabolic activity, cell shape and/or cell polarization and motility. In other words, the majority of cellular activities might be affected. In turn, these activities determine how the individual cell behaves within its population, and thus how it contributes to shaping the population context. These complex and nonlinear feedback mechanisms, which are functional at multiple levels of cellular organization, eventually determine the single-cell distributions of phenotypic properties in a population of cells (FIG. 2). This adaptive multicellular behaviour in *in vitro* conditions may reflect properties of regulatory systems that are normally at work in cells growing in their natural environment of a cell colony or a multicellular organism.

Thus, before it can be stated that stochasticity underlies variability in cellular behaviour, one must be able to dismiss such confounding factors. A stochastic model can nicely fit a distribution of single-cell measurements, even when such distributions are, in reality, determined by a complex set of interactions arising from the population context. The experimental methods used to identify potential stochastic intrinsic noise in gene transcription[32] cannot be easily developed for higher-level cellular activities, which are the eventual outcome of a complex system involving many molecules and interactions. Here, a top-down approach, which tests the presence of predetermining factors in the activity, is more applicable. Analysing whether the activity under study repeatedly occurs in the same cells[17,61], cells of the same lineage[24], cells with similar phenotypic properties[18,19] or cells with a similar population context[6] can reveal such factors (FIG. 3).

Applying cell-to-cell variability

Several recent studies on cell-to-cell variability in mammalian cells seem to confirm the complexity of the deterministic factors involved, and have started to identify predictive aspects of cellular activities that display strong cell-to-cell variability. For instance, the Altschuler laboratory studied how individual cancer cells phenotypically respond in highly variable ways to cancer drugs[79]. It is believed that such non-genetic variability in these responses to drugs adds to the drug resistance of tumours[10]. Interestingly, subpopulation modelling of the heterogeneity observed in sets of signalling molecules in non-perturbed cancer cells allows for predictions of the population-averaged drug-sensitivity of cancer cells. The level of β-catenin, a cytoplasmic cadherin-binding protein that is involved in the cell–cell contact-dependent regulation of cellular growth[7], was found to be a good predictor of drug sensitivity. A study by Sorger and colleagues looked at a part of the genetic circuitry that regulates TRAIL (tumour necrosis factor (TNF)-related apoptosis-inducing ligand)-induced apoptosis, and found that variability in the time between signal and apoptosis was determined mainly by pre-existing variations, most likely in the expression levels of individual components of the signalling pathway[9]. However, a modelling approach and subsequent validation suggested that the level of any individual component in this signalling pathway was insufficient in predicting the time of apoptosis. Instead, it was found that the rate of protein activation involved in the signal relay was highly predictive[9]. Finally, Covert's group studied cell-to-cell variability in TNFα-induced NF-κB shuttling to the nucleus, and found that, upon repeated TNFα stimulation, shuttling was activated tenfold more often in the same cells than expected from a stochastic model[17].

These studies show that pre-existing variations in complex sets of molecular measurements can be highly predictive for a particular phenotypic outcome but that they do not explain the origin of the variation itself. We expect that cell population context, combined with cellular history, plays a significant part in each of these examples. For instance, the sets of molecules analysed might be highly influenced not only by cell adhesion and cell–cell contact signalling (as suggested, for instance, by the predictive power of β-catenin levels in cancer drug sensitivity) but also by nutrient sensing and growth control. These activities are strongly influenced by the population context and history of a cell, such as the local cell density and location on a cell islet edge, or by the proliferation rate of a cell. Indeed, cell density-specific and edge-specific protein expression profiles and proliferation rates have been reported in solid tumours[11,13], and the cell population context-driven switching between proliferative and invasive states

of tumour cells has now been proposed[12]. Furthermore, heterogeneous expression of the transcription factor OCT4 (also known as POU5F1) in stem cell-like cancer cells has been observed in ovarian tumours[80].

Interestingly, in normal stem cell differentiation, cell population context-dependent factors generally have an important role[15] and both stochastic and deterministic causes underlying cell differentiation in early embryogenesis are being discussed[81]. This debate may be resolved in the future by studying cell-to-cell variability over time through applying single-cell tracking and analysis of the cellular context in developing embryos[82], in a similar manner to the approaches outlined above for cells grown in culture. Indeed, it has been shown in multipotent stem cell-like cell lines that differentiation partially depends on local cell density[83], which may determine the currently unexplained heterogeneity in the expression of lymphocyte antigen 6A2–6E1, a differentiation marker in mouse haematopoietic progenitor cells[84].

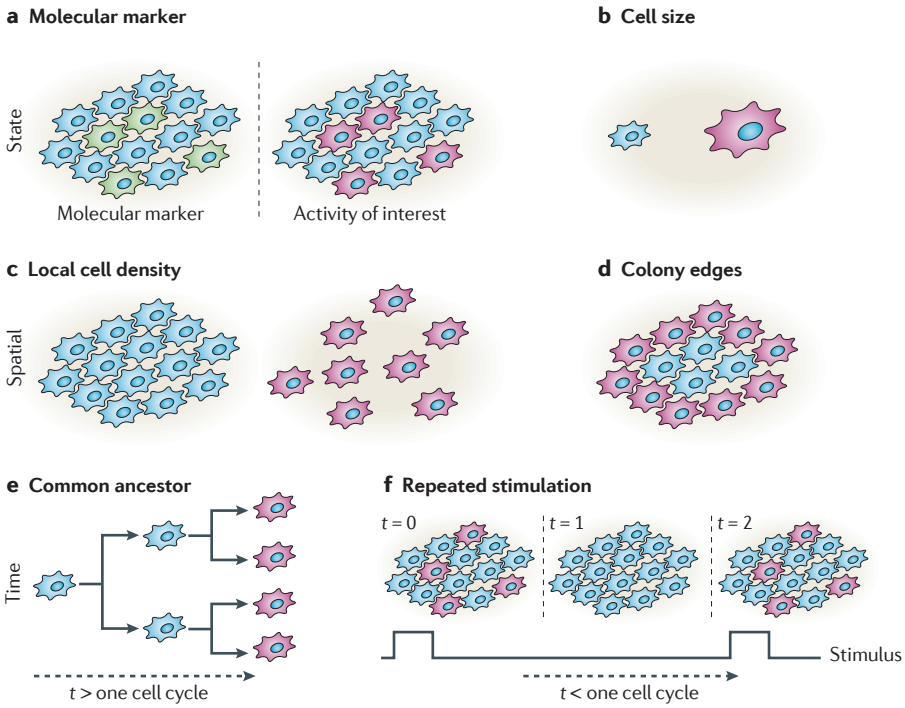


Figure 3 | **Revealing determinism in cell-to-cell variability. a** | The presence of a molecular marker in green cells can be used to predict the activity state of interest in the same cells (pink cells are active). A bistable activity, which is either induced or constitutively present and is active in red cells but inactive in blue cells, is used throughout the figure for simplicity. However, these methods apply equally well to continuous readouts[6,25,84]. **b** | Various cellular state parameters, such as cell size (shown), growth speed and cell cycle state, have been used to explain cell-to-cell variability[6,18,19,26,38]. **c**,**d** | Spatial cell population context parameters such as local cell density (**c**) and location on cell colony edges (**d**) can be another source of deterministic cell-to-cell variability[6,23,83,100]. **e** | Tracing back cell-to-cell variability in time over multiple cell cycles may identify inherited, predetermining factors in cells of the same lineage[24,25]. **f** | Repeated stimulation of the same cells can identify the presence of deterministic factors in seemingly stochastic cell-to-cell variability[17,61]. Note that, in all cases, care must be taken to ensure that the activity of interest does not determine the predictor, thereby creating false predictability. Furthermore, these various deterministic factors are likely to feed back on each other in space and time in a growing cell population.

Impact of cell-to-cell variability

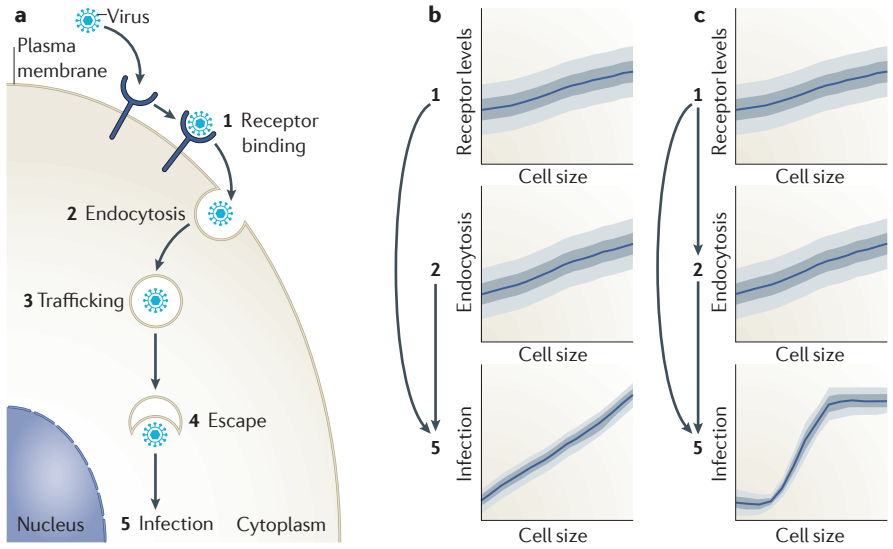
Regulated cell-to-cell variability will have a large impact on many areas of molecular cell biology, as can be illustrated for the process of endocytosis. Certain endocytic ligands take multiple endocytic routes[85,86]. However, as this conclusion was largely based on population-averaged readouts or the analysis of a limited number of single cells, we do not know whether these multiple routes are taken simultaneously within the same single cell or whether the choice of route depends on the population context or phenotypic state of individual cells. The fact that endocytosis and membrane lipid composition are adapted to the population context of an individual cell[6], and that a tight coupling exists between endosome subcellular localization and cell size and shape[68], indicates the existence of uncharacterized regulatory mechanisms. These must be known in order to understand membrane trafficking in the context of cell physiology. Furthermore, it is not clear what the purpose is of adapting endocytosis and membrane lipid composition in single cells to the population context. Is this how cells control their phenotypic state and, if so, which aspects of it?

In addition, if cell-to-cell variability in populations of tissue culture cells is, to a large extent, deterministic, a fundamental flaw exists in several of our current experimental approaches. Perhaps most urgent is to emphasize that we should not average a population of simple tissue culture cells. This is particularly obvious in image-based RNA interference screens but has, thus far, been completely overlooked. In addition, quantitative genetic interactions could act through a population instead of inside a single cell. Describing populations of cells as mixtures of discrete subpopulations goes a step further towards addressing these problems[7,79] but still does not capture the full spectrum of regulated cell-to-cell variability. Thus, also in functional genomics approaches, we will ultimately need to quantify the whole spectrum of single-cell activities in a population of cells and capture the causal interactions of how variations in single-cell activities arise in order to correctly interpret genetic phenotypes and interactions.

Conclusion and perspective

There is increasing experimental evidence that a large part of phenotypic cell-to-cell variability is the result of deterministic regulatory processes. Two classical examples of cell-fate switches in which stochasticity was proposed to play a major part have turned out to be more deterministic at the single-cell level than was previously thought. This opens the door to finding more deterministic aspects in the cell-to-cell variability of these switches and other systems. We find that a common principle emerges, in that cell physiological parameters determined by the cell population context and history are predictive of cell-to-cell variability in a wide range of different activities. Even though the numbers of molecules and molecular interactions inside single cells are inherently noisy, this appears in many cases to have little effect on the eventual cellular activity in which these molecules have a role. Rather, cell-to-cell variability in cellular activities is increasingly found to be the consequence of complex and robust regulatory networks that originate from, and feed back to, the cell population context. This creates a large spectrum of cellular states and activities

Box 2 | **Synergy in virus infection eliminates noise**



Successful virus infection in mammalian cells relies on numerous cellular activities[96] (see the figure, part **a**). These can include mechanisms that determine the levels of receptors and attachment factors on the host cell surface (receptor binding; step **1**), membrane lipid composition, various signalling capacities, endocytic uptake (step **2**), vesicular trafficking (step **3**), membrane barrier escape (step **4**), the microtubule and actin cytoskeleton, nuclear import, and the transcription and translation machinery, as well as metabolic pathways that provide sufficient supplies of nucleotides, amino acids and lipids to create new virus particles[96–98]. Because viruses co-evolved with their host cells, they became highly dependent on a specific set of molecular and cellular processes, or modules, that are required and/or beneficial for infectious entry. Therefore, a virus can only infect (step **5**) a cell that has the appropriate phenotypic state in which these molecular and cellular modules are aligned and active[96,98]. For instance, two modules on which infection depends (receptor levels and endocytosis) can be regulated by cell size (see the figure, part **b**). As a result, these two modules can display synergy, whereby their intrinsic noise (the uncorrelated fluctuations in each graph, indicated by the × 0.5 (dark grey region) and × 1 (light grey region) local standard deviations) is reduced (the local standard deviation becomes smaller in the bottom graph). However, their correlated variability determined by cell size (indicated by the slope of the graphs) is amplified. Furthermore, synergy, or cooperative behaviour, can exist between different modules (see the figure, part **c**). For instance, when a virus binding to its receptor activates endocytosis, a coherent feedforward loop[99] is created. In the hypothetical example (see the figure, part **c**), and based on signal processing theory, this may result in a switch-like behaviour of virus infection efficiency in individual cells as a function of cell size, further enhancing the contribution of determinism in cell-to-cell variability. Such properties of virus infection in tissue culture cells may, in part, reflect the well-known propensity of mammalian viruses to infect specific subsets of cells in the host organism.

in a population of cells, even when these cells do not differentiate.

This now places cell-to-cell variability at the centre of molecular cell biology, generating a number of research questions for the future. At the phenotypic level, cell-to-cell variability can be used to discover new regulatory mechanisms, which will increase our understanding of how cellular activities are embedded in the physiology of a cell. At the molecular level, it will be important to understand the mechanisms by which cells cancel out intrinsic noise while amplifying regulated cell-to-cell variability (see FIG. 1d and BOX 2 for mechanisms that employ synergy in virus infection). To reveal these mechanisms, studies of cell-to-cell variability will eventually need to include aspects of the regulated subcellular localization, and transient activation and inactivation, of molecular machinery components[43,87]. These types of analyses must now be done in large numbers of individual cells, and should be combined with measurements of single-cell transcription rates and protein levels, and with quantitative analyses of single-cell parameters of population context and physiological state. Such a comprehensive quantitative analysis of a cellular system is challenging, but certainly possible with current technologies. However, even without a full understanding of the underlying molecular mechanisms, the amount of determinism in the cell-to-cell variability of complex cellular activities can be studied using top-down approaches and we can arrive at more accurate predictive models[3,6,9]. These top-down models can then be used as guidelines to uncover the relevant molecular circuits, enabling us to attain quantitative molecular models that accurately predict and simulate the variable single-cell behaviour within a population of cells.

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Competing interests statement

The authors declare no competing financial interests.

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