

What's all the noise about developmental stochasticity?

Andrew C. Oates*

Summary

In October 2010, researchers from diverse backgrounds collided at the historic Cumberland Lodge (Windsor, UK) to discuss the role of randomness in cell and developmental biology.

Organized by James Briscoe and Alfonso Martinez-Arias, The Company of Biologists' workshop was the latest in a series of meetings aimed at encouraging interdisciplinary interactions between biologists. This aim was reflected in talks at this workshop that ranged from the tissue to the cellular scale, and that integrated experimental and theoretical approaches to examining stochastic behavior in diverse systems.

Key words: Time lapse microscopy, Stem cells, Signaling, Population heterogeneity, Incomplete penetrance, Gene expression noise

Introduction

The beautiful dynamic order of developing biological systems attracts our attention, yet many of the constituent parts of these systems are inherently stochastic. The buzzing noisiness of molecular components is, at first glance, difficult to reconcile with the apparent high reproducibility of embryonic development. This tension between randomness and order raises questions such as how is disorder brought under control during development and, by contrast, when is it used to generate pattern?

Canalization is a term that was coined by C. H. Waddington to describe biological robustness (Waddington, 1942), i.e. the ability of a population to develop the same phenotype despite variation in environment or genotype. Although environmental variation can arise from external fluctuations in, for example, temperature, pH and nutrients, it can also include the molecular noise of a system's biochemistry, which is subject to random thermal fluctuation. Importantly, thermal fluctuations can govern a regulatory system when the number of participating molecules is low (Delbrück, 1940). In developing embryos, cell numbers are low, and the timescales over which key decisions are made can be short; consequently, the number of molecules involved in such decisions is often correspondingly low. With low numbers come strong fluctuations. Thus, whenever low molecule numbers and/or small system sizes exist in development, each developmental decision is influenced by a roll of the molecular dice.

This finite number effect on noise is not restricted to molecules, however, and whenever systems select from a low number of discrete outcomes, the effect of noise becomes evident. For example, when cells divide, low numbers of discrete organelles must be segregated. If a low number of stem cells compete in a niche, the resulting daughter populations may show strong biases. At a

macroscopic level, genetic bottlenecks are a well-known example of the outcome of the selection of a low number of surviving individuals. However, finite number effects are not the only source of noise for a developing embryo; migrating cells, for example, can show a stochastic component to their movement, which is well described by a random walk (Pezeron et al., 2008). As reflected by the talks at this workshop, only relatively recently has interest and technical capability combined to give the first molecular descriptions of the roles played by noise in metazoan development (see Huang, 2009; Kilfoil et al., 2009; Losick and Desplan, 2008; Raj and van Oudenaarden, 2008).

Understanding incomplete penetrance

Incomplete penetrance, in which genetic mutation results in a variable phenotype in a population, was one of the earliest observed instances of stochasticity in development, first noticed in 1925 (Romaschoff, 1925; Timofëeff-Ressovsky, 1925). In his plenary lecture, Alexander van Oudenaarden (Massachusetts Institute of Technology, MA, USA), whose laboratory has focused on understanding the origins and consequences of stochastic gene expression, initially in microbial systems and more recently in metazoans, demonstrated how counting single mRNA molecules (Raj et al., 2008) in fixed *C. elegans* embryos can be used to examine how noisy transcription influences developmental events and to provide a mechanistic explanation for the phenomenon of incomplete penetrance (Raj et al., 2010). van Oudenaarden's group looked at the process of intestinal cell specification in *C. elegans*, which is mediated by a small genetic regulatory network (GRN) of interacting transcription factors (Fig. 1). In this GRN, *skn-1* is maternally provided to the E cell (which gives rise to all 20 intestinal cells) and activates a small network of transcription factors that control the transcription of *elt-2*, which in turn controls the expression of the intestinal differentiation program. *skn-1* mutants have an incompletely penetrant phenotype, as most lack intestinal cells. By counting the number of *elt-2* transcripts, van Oudenaarden's group found that whereas some *skn-1* mutant embryos express *elt-2* at near wild-type levels, most have no *elt-2* expression, producing a bimodal distribution (Raj et al., 2010). One of the direct regulators of *elt-2*, *end-1*, shows a strong unimodal variability in transcript number. Further analysis showed that the bimodal *elt-2* distribution appears to derive from a threshold effect: in those cells that accumulate enough END-1 to initiate *elt-2* expression, the auto-regulatory action of ELT-2 locks it on. Because reducing the activity of a key chromatin regulator in a *skn-1* mutant background partially rescued *end-1* expression and restored *elt-2* transcript numbers, the group propose that chromatin structure affects gene expression noise in this system, while redundancy in the GRN contributes to the timing and levels of *elt-2* transcription (Raj et al., 2010). Thus, van Oudenaarden showed that the function of the intestinal GRN is to buffer stochastic variability in gene expression; loss of elements of this network produces pronounced phenotypic variation that reveals the inherent stochasticity of gene expression.

Population heterogeneity in signal transduction

Many of the participants at the workshop were interested in the phenomenon of population heterogeneity, where a system's average behavior does not reflect the average behavior of the system's components. Experimental techniques in which cells or embryos are pooled together, such as in western blotting or microarray screens,

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany.

*Author for correspondence (oates@mpi-cbg.de)

can give quantitative results of the system's average behavior in response to a stimulus. However, experiments that examine each member of the population, such as fluorescence-activated cell sorting (FACS) or single-cell quantitative (q)PCR can reveal that cell-to-cell heterogeneities underlie this average response (Fig. 2). Time-lapse imaging with single-cell resolution can also reveal rich heterogeneity in temporal behavior. This diversity of response in an apparently identical population to an apparently uniform stimulus raises many questions. Are the members of the population really uniform? What sort of microscopic diversity is present? How are the diverse heterogeneous states related to each other? What biological function might the population structure have?

Suzanne Gaudet (Harvard Medical School, MA, USA) investigates in her work the variability in cellular responses to the apoptosis-inducing TRAIL ligands. Human cancer cells have a strongly heterogeneous response to such ligands: some cells die within 1 hour, others 8-12 hours later, whereas others live indefinitely (Spencer et al., 2009). Using live-cell imaging, in which fluorescent reporters of apoptosis signaling components were monitored, Gaudet described how the timing of apoptosis in sister cells was highly correlated, independent of the cell cycle stage, and how this correlation was gradually dampened by ongoing protein synthesis. These results indicate that the variability was not due to short-term fluctuations, e.g. noise in the signaling response, but rather to a slow fluctuation in the levels of key apoptosis proteins, which she termed transient heritability. Population heterogeneity on this time scale (hours to days) (Huang, 2009) may be an extremely important factor in developmental events of longer duration, and in regeneration or cancer. To describe these results, Gaudet introduced a detailed molecular network model with ~60 differential equations and ~150 parameters describing synthesis and degradation rates (Albeck et al., 2008). Such complexity is not for the faint-hearted; this model was rigorously compared with data, and a systematic procedure for calibration was developed (Kim et al., 2010).

Jeremy Gunawardena (Harvard University, MA, USA) described epidermal growth factor (EGF) signaling in fixed mouse fibroblasts using quantitative immunofluorescence to measure the phosphorylation of the EGF receptor and the downstream kinase ERK. Although as a population, the levels of these phospho-proteins rise smoothly with increasing dose and time after EGF stimulation, single-cell quantitation revealed a bi-modal distribution of activated cells that changed with dose and time (Y. Xu and J. Gunawardena, unpublished). Thus, the number of activated cells in the population increased as the dose of EGF increased (see Fig. 2B). Gunawardena's group developed a modeling approach inspired by quantitative pharmacology (Black and Leff, 1983) that simplifies the underlying molecular processes into two modules: a receptor pool with a hyperbolic dose response coupled to a transduction network with a non-linear threshold. This model fits the experimental data, enabling the systems-level properties of the modules to be directly quantified. Such a general modular approach (Mallavarapu et al., 2009) could be extremely useful for understanding aspects of development when a complete parts list is not known or when it is difficult to constrain molecular parameter values.

Transcriptional noise at the onset of multicellularity

It is well known from microbial studies that isogenic cells may not have the same phenotype. Studies of gene expression noise in microorganisms have revealed a plethora of noise-related effects (Eldar and Elowitz, 2010). One organism that changes its behavior from microbial to metazoan within its life cycle is the slime mold

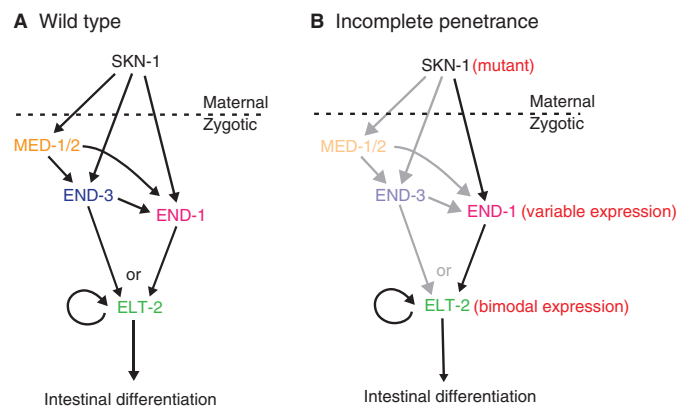


Fig. 1. The genetic regulatory network controlling intestinal specification in *C. elegans*. (A) Wild-type genetic regulatory network (GRN). SKN-1 is maternally provided to the E cell (which gives rise to all 20 intestinal cells) and activates the transcription of *med-1/2*, *end-3* and *end-1*. MED-1/2 and END-3 subsequently activate *end-1* together with SKN-1. Finally, END-1 and END-3 both redundantly activate transcription of the master regulatory transcription factor ELT-2, which controls the expression of hundreds of intestinal differentiation genes. (B) *skn-1* mutant GRN. The *skn-1* alleles used by Raj et al. (Raj et al., 2010) do not activate *med-1/2* or *end-3*, and the resulting *end-1* expression is highly variable. Expression of *elt-2* is bimodally distributed, probably owing to a threshold effect due to its autoregulation. See text for details. Modified, with permission, from Raj et al. (Raj et al., 2010).

Dictyostelium, which is studied by Johnathan Chubb (University of Dundee, UK), who presented his group's analysis of in vivo transcriptional dynamics by GFP tagging of the developmentally regulated *ecmA* gene (Stevens et al., 2010). Their approach allowed them to visualize nuclear foci of around 10-20 *ecmA* transcripts, the levels of which fluctuate (pulse) in response to the stalk-inducing signals DIF (a cAMP antagonist) and cAMP. By counting these stochastic pulses, Chubb's group showed that the response to increasing signal concentration and duration was an elevation in the number of cells in the population that pulsed, but only a mild increase in the time spent in the active state or in the pulse intensity. These results indicate a digital response to a graded signal (Fig. 2C), much like the heterogeneous EGF responses described above. Chubb also showed that the time spent in the active state by a cell is a property shared by its daughters after division, an inheritance pattern that requires histone 3 K4 methylation (Muramoto et al., 2010). Thus, even though the actual pattern of pulses was apparently stochastic in the short term, the integrated rate of pulsing fluctuated over timescales longer than the cell cycle.

To synch or not to synch? That is the signal question

Noisy transcription can yield noisy levels of important signaling molecules, and in some developmental settings, this could pose a problem for accurate and precise patterning. To overcome this, a cell's dynamics could be synchronized with its neighbors to deliver a coherent signal, e.g. when a signal is oscillating. Conversely, if an oscillating signal were potentially damaging when synchronized, a mechanism must exist to prevent this coherent state from being established. Finally, signaling noise may be useful to prevent a patterning system becoming locked in a suboptimal configuration. Three presentations at the workshop highlighted these different cases of noise in cellular signaling.

Andrew Oates (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany) presented his group's studies of the zebrafish segmentation clock, which operates in the cells of the presomitic mesoderm (PSM) and provides the temporal cues for the rhythmic and sequential formation of somites, possibly via a transcriptional network involving negative feedback (Dequeant and Pourquie, 2008; Schroter and Oates, 2010). Previous single-cell studies have suggested that the cellular oscillations of the clock are unstable and noisy (Masamizu et al., 2006). Synchronization of this oscillating cell population via Delta-Notch signaling is crucial to suppress the noise and allow effective segmentation of the embryo (Riedel-Kruse et al., 2007). Oates discussed zebrafish Delta-Notch signaling mutants, in which the period of the segmentation clock is changed (Herrgen et al., 2010). To understand this result, Oates used a theory describing the PSM as a population of coupled cellular oscillators, in which the collective period of the population is altered as a function of the time delays that arise from the synthesis and trafficking of the coupling molecules (Morelli et al., 2009). By fitting the theory to data on period change and oscillating gene expression obtained from Notch signaling mutants, they were able to estimate the autonomous period, and the delay and strength in the coupling (Herrgen et al., 2010). Thus, although the main function of Delta-Notch signaling in the embryo is to synchronize noisy oscillating PSM cells, the time delays introduced by coupling these cells with large macromolecules such as Delta cause the period of oscillations to change.

Mike White (University of Liverpool, UK) presented recent work from his group on the oscillatory dynamics of NF- κ B signaling in mouse cells, which appear to be essential for its functions in the inflammatory response. He described how the frequency of these oscillations determines the spectrum of target genes that are induced by NF- κ B (Ashall et al., 2009). One such target is tumor necrosis factor (TNF), which is also the ligand that initiates signaling via NF- κ B by releasing it from inhibitory I- κ B subunits that anchor NF- κ B in the cytoplasm, thus allowing its translocation to the nucleus. NF- κ B also regulates I- κ B and I- κ B ϵ transcription in delayed negative-feedback loops that drive oscillations in NF- κ B translocation by trapping NF- κ B once again in the cytoplasm (Paszek et al., 2010a). However, if all cells in a tissue were to synchronize their NF- κ B signaling, the tissue might produce supra-physiological bursts of TNF, potentially leading to shock and death of the organism. White's analysis of the dual delays caused by the I- κ B and I- κ B ϵ -mediated negative-feedback loops show that they cause an increase in noise in the overall timing of the NF- κ B autoregulatory loop, thereby generating differences in oscillation period between cells (Paszek et al., 2010b) and preventing the synchronous release of TNF.

Buzz Baum (University College London, UK) described his group's studies of the role of stochastic signaling in determining the regular spacing of bristle precursor cells on the epithelium of the *Drosophila notum* (Cohen et al., 2010a). Current models of this arrangement invoke Delta-Notch-mediated lateral inhibition by nearest-neighbor cells. Baum described how their time-lapse imaging of bristle precursor cell specification in living embryos allowed them to observe how the final position of precursor cells is refined over time. When they simulated this process computationally by modeling signaling via lateral inhibition that was both strictly near-neighbor and continuous in time, they could not reproduce either the refinement process or the final steady-state pattern. However, when they incorporated signaling via the dynamic and intermittently forming basal protrusions that form between epithelial cells beyond their nearest neighbors, their model more closely fitted what they had observed (Cohen et al., 2010a). Experimental analysis then revealed

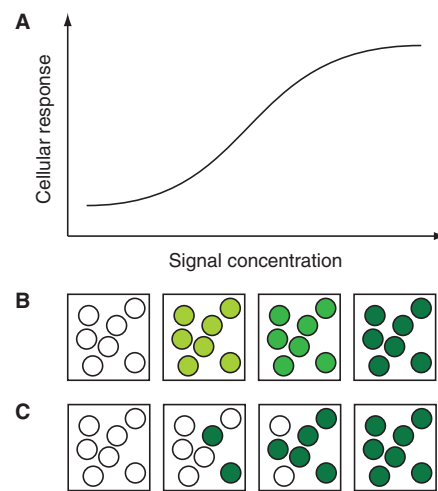


Fig. 2. Population heterogeneity in signaling response. (A) Plot of the dependence of a marker of cellular signaling response to the concentration of the signal, averaged over the entire cell population. (B) Cells increasing their response uniformly to the increase in signal concentration (graded green shading indicates a graded response). (C) Cells stochastically switching on an all-or-none response with higher probability at higher signal concentration.

that protrusion formation is indeed required for proper patterning, indicating that intermittently growing and retracting cellular protrusions might represent a structured noise in signaling that helps to refine a spacing pattern without cells becoming trapped in suboptimal patterns (Cohen et al., 2010b).

The slippery slope of embryonic gradients

Patterning of embryonic tissues by morphogen gradients is a classic concept in developmental biology. The French flag model of Lewis Wolpert describes the activation of abutting domains of target genes across a field of cells in a concentration-dependent manner by a morphogen released from one edge (Wolpert, 1969). Boundaries between these domains subdivide the tissue, and can also act as local centers to further elaborate the patterning: their precise position is therefore important. Noise could interfere with reliable patterning via a gradient in several ways, through: temporal fluctuations in signaling molecule production; heterogeneity of receptors and transduction proteins in target cells; and movement and division of cells within the gradient (Wartlick et al., 2009). How precise boundaries are nonetheless produced is the subject of current debate, and three presentations dealt with various aspects of the problem.

Jim Smith [National Institute for Medical Research (NIMR), London, UK] presented his group's work investigating how gradients of transforming growth factor (TGF) family members cause reliable patterning in the early vertebrate gastrula. At this stage, restricted and well-defined domains of *no tail* and *gooseoid* are formed in response to Nodal/Activin signals, but it is not understood how the size and shape of the domain is so precisely regulated. He focused on the response of the target cells, as measured by a fluorescent sensor of Nodal/Activin signaling (Harvey and Smith, 2009). Data from both *Xenopus* and zebrafish embryos showed that the resulting gradient of nuclear fluorescence is highest on the dorsal side, as expected, but was very noisy, and the question was posed as to how the embryo could make use of this signal to reliably position the *no tail* and *gooseoid* domains (Harvey et al., 2010; Saka et al., 2007)?

Work from James Briscoe's laboratory (NIMR, London, UK) suggests how a tissue might take an imprecise gradient and generate more precise patterns from it. His group has explored the patterning of the mouse vertebrate neural tube by a gradient of sonic hedgehog (Shh). Shh is synthesized in the ventral neural tube, and induces the expression of three key transcription factors in sequential domains away from its source: Pax7, which is expressed most dorsally; Nkx2.2, most ventrally; and Olig2, which is located in between (Ribes and Briscoe, 2009). Briscoe discussed his group's quantitative analysis of the relationship between the Shh protein gradient and these expression domains, which showed that the amplitude of the Shh gradient increases over time, but its decay length remains approximately constant. Furthermore, the positions of the boundaries between the expression domains change in time, but these changes do not correlate with changes in the Shh gradient. Importantly, when different embryos are compared, the precision of the gradient is lower than the precision of the expression boundaries (Dessaud et al., 2010; Dessaud et al., 2007). Combined, these findings suggest that additional processes in the target tissue must actively contribute to the final pattern.

From their experimental results, Briscoe and colleagues propose that a feedback loop in the Shh signal transduction cascade generates temporal adaptation to persistent Shh signaling, and that the mutually repressive interactions among the transcription factor targets of Shh contribute to the sharpening of the boundaries (Dessaud et al., 2010). A GRN model of this system based on these data recapitulates several key features, such as time integration of the Shh signal, which produces the three expression states sequentially, and a strong robustness to noise. These results suggest that over longer timescales, the network in the target tissue, rather than the ligand concentration, has a dominant role in determining gene expression levels (Jaeger et al., 2008).

Similar questions of gradient and target precision have motivated the group of Marcos Gonzalez-Gaitan (University of Geneva, Switzerland) (Bollenbach et al., 2008; Kicheva et al., 2007), who described the role of another TGF family member, the *Drosophila* Dpp protein, in precisely controlling wing size via its signaling gradient in the corresponding imaginal disc. He presented new work based on quantitative long-term time lapse imaging of disc growth on the dynamic features of the gradient that cells use to determine their rate of division (O. Wartlick and M. Gonzalez-Gaitan, unpublished).

Pluripotent noise from the early mouse embryo

Pluripotent mouse embryonic stem (ES) cells are derived from the inner cell mass (ICM) of the mouse blastocyst, and can be maintained in culture or differentiated into various cell types in vitro. Their properties make them an attractive tool for investigating early mammalian development, differentiation and, more recently, stem cell dynamics. Importantly, although ES cells can contribute to all tissues of an embryo on transplantation into a host blastocyst, less than one in ten transferred cells actually does (Wang and Jaenisch, 2004), indicating that cultured ES cells are a heterogeneous population. Given the importance of ES cells for studying cell and developmental biology, this heterogeneity has practical as well as theoretical implications.

A dynamic basis for ES cell heterogeneity was explored by Joshua Brickman (University of Edinburgh, UK), who described his group's analysis of *Hex* gene expression, which marks one of the two ICM-derived lineages: the primitive endoderm. *Hex* expression was detected using a novel *Hex*-tandem IRES-*Venus* YFP construct, and was found to be variably expressed in ES cells (Canham et al., 2010). Remarkably, when the group used FACS to enrich for ES

cells with high or low Venus levels, they found that each ES cell population could regenerate the full range of Venus expression after ~24 hours. Thus, the culture appeared to contain a dynamic equilibrium of different cell types that under ES cell culture conditions were inter-convertible. Nevertheless, by transplanting cells immediately after FACS enrichment into host blastocysts, a clear difference in the populations was observed: the Venus-positive cells contributed preferentially to derivatives of the primitive endoderm and the Venus-negative cells contributed exclusively to epiblast-derived cells. In embryoid bodies, Venus-positive cells segregated to the inside, and the Venus-negative cells to the outside, reflecting the relative positions of primitive endoderm and epiblast in the early mouse embryo. Importantly, the Venus-positive cells persisted when FGF signaling was blocked, suggesting that the previously identified 'ground state' of ES cells (Ying et al., 2008) may also consist of several interchanging components. Thus, the transient states that ES cells cycle through in culture (Kalmar et al., 2009; Kobayashi et al., 2009) are likely to be relevant in vivo, and may represent the priming or availability of cells to be recruited into the different lineages of the early mouse embryo.

The mouse blastocyst also displays stochastic distribution of early gene expression (Dietrich and Hiiragi, 2007; Ralston and Rossant, 2008) and its early segregation into epiblast ectoderm and primitive endoderm is due to cell sorting subsequent to the initial noisy assignment of cell fates (Plusa et al., 2008; Yamanaka et al., 2010). However, analysis of this early developmental phase is hindered by a paucity of markers with which to follow fate and sorting in live embryos. Takashi Hiiragi (Max Planck Institute of Molecular Biomedicine, Münster, Germany) presented results of a Venus YFP promoter-trap screen designed to identify genes expressed in the blastocyst and to characterize their dynamics during embryonic patterning. Five out of the 22 positive lines exhibit heterogeneous expression of the trapped gene, suggesting that a significant proportion of the genome is involved in this early stochastic cell fate assignment. Without appropriate cell sorting, heterogeneous fate choices would not build an embryo, and Hiiragi also presented a mathematical model to describe the shapes of the early cells and the forces acting upon them (Honda et al., 2008). In simulating the cavitation of the blastocyst, the model successfully generated the characteristic bimodal distribution of cell sizes observed in the embryo. Thus, by combining fate and movement data from the Venus-trap lines with the vertex model, it may be possible to understand how stochastic fate choices and subsequent morphogenesis can reliably allow the early mouse embryo to self-organize.

Hematopoietic stem and progenitor cell stochasticity

Hematopoietic stem cells (HSCs) are multipotent cells that can give rise to all blood cell types, and a single HSC can completely reconstitute the adult hematopoietic system of a deficient host (Osawa et al., 1996). As such, HSCs are important as a model for stem cell and lineage differentiation, and for therapeutic studies into treating immunodeficiency and cancer. However, HSCs are extremely rare, and existing HSC isolation procedures result in molecularly and behaviorally heterogeneous cell populations (Schroeder, 2010). Fresh insight into this heterogeneity could come from Timm Schroeder's (Helmholtz Zentrum, München, Germany) spectacular software called Timm's Tracking Tool, which allows individual cells to be tracked over days to weeks in culture (Eilken et al., 2009) with images acquired every few minutes using multiple marker channels. The resulting annotated movies are used to

reconstruct hundreds of cellular pedigrees in which the timing and context of a particular cell behavior can be observed (Eilken et al., 2009; Rieger et al., 2009).

One unresolved issue that has been addressed using this tool is whether cytokine signaling to hematopoietic progenitor cells (HPCs), the lineage-restricted daughters of HSCs, is instructive, causing a cell to follow a particular lineage, or selective, allowing cells that have made an earlier lineage choice to survive. To address this, Schroeder's group measured the frequency of cell death in over 300 clonal colonies for each cytokine (Rieger et al., 2009). Over 85% of exclusively granulocyte- or macrophage-producing colonies grew without any cell death, thus most of the cells in the experiment had been directly instructed to follow a particular lineage. The group also investigated the developmental origin of hematopoietic cells by isolating ES cell-derived mesodermal cells, which were cultured to determine whether and at what point individual hematopoietic cells were observed (Eilken et al., 2009). Adherent, sheet-like endothelial cells were filmed giving rise to free-floating hematopoietic cells during a transient window from days 6 to 7 in culture. The power of the software is evident by the numbers: in 25 experiments, the generation of over 4.3×10^5 individual cells in more than 10,000 colonies was recorded and the hematopoietic transition was observed in 114 of these colonies. The same transition was seen in primary cells isolated from mouse embryos, suggesting the *in vivo* relevance of the observations. These approaches illustrate the necessity for and power of following single cells through long-term behaviors, and they also highlight the sobering challenge that lies before developmental biologists to follow statistically significant numbers of cells with a similar resolution within a developing organism.

Stem cell sisters are sometimes symmetric

The prevailing concept of an adult stem cell niche emphasizes the role of the local micro-environment in hosting the stem cell and that each stem cell steadily generates a clone of progeny by asymmetric cell division that makes up a defined part of the resulting tissue mosaic (Yamashita et al., 2010). Ben Simons (University of Cambridge, UK) discussed work arguing against this prevailing view, proposing that this is just one of three possible ways to maintain a tissue at homeostasis through stem cell divisions. The other two possibilities can be termed population asymmetries, in which there is no invariant asymmetric cell division but, rather, equivalent stem cells that divide stochastically with a certain probability either to self-renew or to give rise to committed daughters.

In tissues maintained by invariant division asymmetry, the composition of clones is expected to remain fixed in size and number. In the stochastic model, cells follow neutral drift dynamics in which continual clonal loss is compensated for by the expansion of neighboring clones, i.e. an ever-decreasing number of clones of an ever-increasing size (Lopez-Garcia et al., 2010; Snippert et al., 2010). This long-term clonal size distribution acquires a simple scaling form, which can be used to discriminate between different fate patterns. Such clone behavior may sound counterintuitive, but was tested *in vivo* using a genetic cell marker system termed *Confetti*, which results in the individual labeling of all the cells in a tissue with different sets of fluorescent protein variants (Snippert et al., 2010). As Simons showed, labelled mouse intestinal crypts provide compelling (and beautiful) evidence that although each crypt is composed of several clones shortly after labeling, with time, the number of clones decreases while the size of the remaining clones increases (Fig. 3). The size distribution of these clones fits

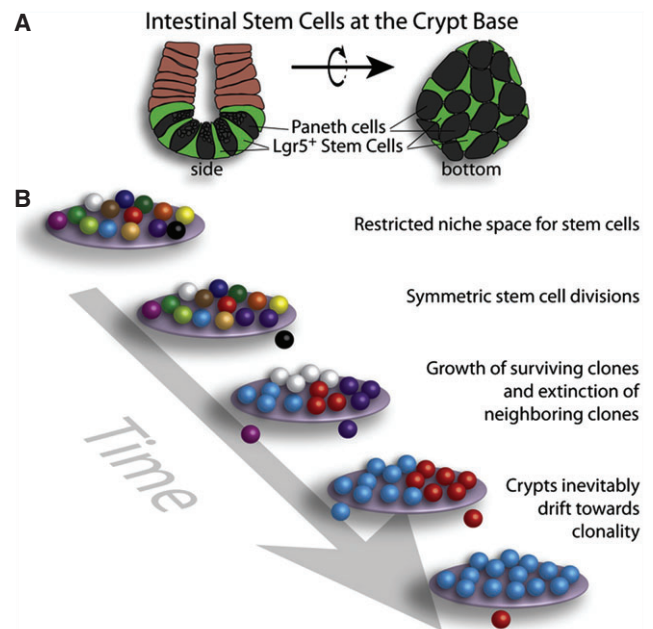


Fig. 3. Neutral drift dynamics of symmetrically dividing stem cells in the intestinal crypt. (A) A schematic of the mouse intestinal crypt base, showing the approximate location and number of Paneth cells and the *Lgr5*⁺ stem cells that give rise to the differentiated cells of the crypt and villi (in brown). (B) A representation of the effects of neutral drift dynamics on an individually marked population of stem cells (different colors) in the crypt base. Over time, as stem cells are lost and replaced by their neighbors through stochastic symmetric divisions, the number of clones becomes smaller and the size of the remaining clones becomes correspondingly larger. Eventually, the crypt becomes monoclonal. Reproduced, with permission, from Snippert et al. (Snippert et al., 2010).

with the neutral drift dynamics theory, allowing the stem cell replacement rates to be estimated (Snippert et al., 2010). Remarkably, similar clonal distributions have also been observed in the skin (Clayton et al., 2007; Doupe et al., 2010) and the testes (Klein et al., 2010). At the moment, it seems hard to reconcile these findings with existing studies on asymmetric division. Clearly, individual stem and progenitor cells in different systems can be imaged undergoing asymmetric divisions (Yamashita et al., 2010), but some of these situations might now be re-examined with a statistical eye on the consequence of other modes of division. In particular, it will be exciting to learn which biochemical mechanisms constitute the proposed stochastic fate switches that give rise to homeostatic tissue maintenance at the population level.

Stochasticity and cellular mechanics

The noise inherent in small numbers of molecules is also an important consideration for understanding mechanical processes, such as cell division, cell shape and cell movement. In a presentation by François Nédélec (European Molecular Biology Laboratory, Heidelberg, Germany) the stochastic process of microtubule growth and shrinkage, termed dynamic instability, was investigated during spindle formation using a simulation where individual microtubule subunits, as well as associated proteins such as motors, were tracked. Using values for the biochemical interactions that are well constrained by data, the model reproduces key aspects of catastrophe, when the microtubule starts to shrink, and the self-organizing behavior of the mitotic spindle (Brun et al., 2009). A

strength of the model is that individual protein components or activities can be reduced or increased, and the resulting dynamic structure can be directly compared with experimental data.

Cytoskeletal dynamics and organization was discussed by Thomas Lecuit (L'Institut de Biologie du Développement de Marseille Luminy, Marseille, France), who reported work from his group investigating how cell shape results from the interaction between cytoskeletal force generation and cell adhesion in the embryonic epithelia of *Drosophila*. Analysis of high-resolution time-lapse microscopy revealed that morphogenetic cell junction shrinkage is preceded by the polarized flow of actomyosin pulses towards the junctions, and the flow is oriented towards polarized distribution of E-cadherin complexes (Rauzi et al., 2010). Thus, the steady-state distribution of Myosin II motors does not reflect the fluctuating interactions between cytoskeletal contractile flows and cell adhesion complexes present in epithelial morphogenesis. Perhaps these fluctuations are a mechanical equivalent of the structured noise discussed by Buzz Baum, and prevent the tissue from becoming trapped in a suboptimal packing arrangement.

Ray Goldstein (University of Cambridge, UK) described studies of the swimming behavior of the photosynthetic *Chlamydomonas*, which uses two beating flagella to propel itself. By combining high-speed imaging of individual cells held on a micropipette with a tracking microscope to follow individual freely swimming cells over long intervals, the Goldstein group could show that individual cells can switch stochastically between three beat behaviors: synchronous, brief phase slips where the two flagella move out and back into sync; and a fully asynchronous drifting of the flagellar beats. The dynamics of switching perfectly yields the distribution of behaviors observed in the population (Polin et al., 2009); thus, the population structure at any moment is generated by the dynamics of the individual swimmers. The purpose of such stochastic switching is related to locomotory function. *Chlamydomonas* swims straight ahead when beating synchronously, but turns when the beat becomes asynchronous, moving in a new direction when normal service resumes. By tuning switching frequency, *Chlamydomonas* could effectively search for and adapt to changing resources in the environment. The themes of this work, relating population heterogeneity and individuality, show a parallel to the pioneering analysis of stochasticity in *E. coli* run and tumble swimming (Spudich and Koshland, 1976). Although the stochasticity of beat switching is currently described at the level of the entire cell, analysis of *Chlamydomonas* mutants with aberrant swimming promises to reveal the genes and underlying stochastic biochemistry of the switching mechanism.

Conclusion

Key themes at the workshop were the dissection of population properties caused by single cell heterogeneity, and the distinction of population noise resulting from longer time-scale fluctuations, versus temporal noise resulting from the stochastic fluctuations in gene expression. What was clear is the importance of single object dynamics, regardless of the object and system, as only with the time correlations of individual components can the relationships between parts and wholes be reliably understood in stochastic systems. The technical ability to perform single-object tracking, whether this be molecules in a cell, cells in an embryo or even birds in a flock, is therefore a key step in understanding how the structure of noise at lower levels affects the behavior of the system at higher levels. This poses an exciting challenge for developmental biologists, because obtaining time series at single cell resolution or lower from within embryos with the duration and quality available in microbial or cell culture systems remains difficult. Nevertheless, advances in imaging

technology and increasing collaboration between experimentalist and theorist, facilitated by workshops such as this, will drive exploration into the stochastic side of life.

Acknowledgements

A.C.O. is supported by the Max Planck Society and the European Research Council under the European Communities Seventh Framework Programme.

References

- Albeck, J. G., Burke, J. M., Spencer, S. L., Lauffenburger, D. A. and Sorger, P. K. (2008). Modeling a snap-action, variable-delay switch controlling extrinsic cell death. *PLoS Biol.* **6**, 2831-2852.
- Ashall, L., Horton, C. A., Nelson, D. E., Paszek, P., Harper, C. V., Sillitoe, K., Ryan, S., Spiller, D. G., Unitt, J. F., Broomhead, D. S. et al. (2009). Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. *Science* **324**, 242-246.
- Black, J. W. and Leff, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B Biol. Sci.* **220**, 141-162.
- Bollenbach, T., Pantazis, P., Kicheva, A., Bokel, C., Gonzalez-Gaitan, M. and Julicher, F. (2008). Precision of the Dpp gradient. *Development* **135**, 1137-1146.
- Brun, L., Rupp, B., Ward, J. J. and Nedelec, F. (2009). A theory of microtubule catastrophes and their regulation. *Proc. Natl. Acad. Sci. USA* **106**, 21173-21178.
- Canham, M. A., Sharov, A. A., Ko, M. S. and Brickman, J. M. (2010). Functional heterogeneity of embryonic stem cells revealed through translational amplification of an early endodermal transcript. *PLoS Biol.* **8**, e1000379.
- Clayton, E., Doupe, D. P., Klein, A. M., Winton, D. J., Simons, B. D. and Jones, P. H. (2007). A single type of progenitor cell maintains normal epidermis. *Nature* **446**, 185-189.
- Cohen, M., Georgiou, M., Stevenson, N. L., Miodownik, M. and Baum, B. (2010a). Dynamic filopodia transmit intermittent Delta-Notch signaling to drive pattern refinement during lateral inhibition. *Dev. Cell* **19**, 78-89.
- Cohen, M., Baum, B. and Miodownik, M. (2010b). The importance of structured noise in the generation of self-organizing tissue patterns through contact-mediated cell-cell signalling. *J. R. Soc. Interface* (in press).
- Delbrück, M. (1940). Statistical fluctuations in autocatalytic reactions. *J. Chem. Phys.* **8**, 120-124.
- Dequeant, M. L. and Pourquie, O. (2008). Segmental patterning of the vertebrate embryonic axis. *Nat. Rev. Genet.* **9**, 370-382.
- Dessaud, E., Yang, L. L., Hill, K., Cox, B., Ulloa, F., Ribeiro, A., Mynett, A., Novitsch, B. G. and Briscoe, J. (2007). Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism. *Nature* **450**, 717-720.
- Dessaud, E., Ribes, V., Balaskas, N., Yang, L. L., Pierani, A., Kicheva, A., Novitsch, B. G., Briscoe, J. and Sasai, N. (2010). Dynamic assignment and maintenance of positional identity in the ventral neural tube by the morphogen sonic hedgehog. *PLoS Biol.* **8**, e1000382.
- Dietrich, J. E. and Hiiragi, T. (2007). Stochastic patterning in the mouse pre-implantation embryo. *Development* **134**, 4219-4231.
- Doupe, D. P., Klein, A. M., Simons, B. D. and Jones, P. H. (2010). The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate. *Dev. Cell* **18**, 317-323.
- Eilken, H. M., Nishikawa, S. and Schroeder, T. (2009). Continuous single-cell imaging of blood generation from haemogenic endothelium. *Nature* **457**, 896-900.
- Eldar, A. and Elowitz, M. B. (2010). Functional roles for noise in genetic circuits. *Nature* **467**, 167-173.
- Harvey, S. A. and Smith, J. C. (2009). Visualisation and quantification of morphogen gradient formation in the zebrafish. *PLoS Biol.* **7**, e1000101.
- Harvey, S. A., Tumpel, S., Dubrulle, J., Schier, A. F. and Smith, J. C. (2010). No tail integrates two modes of mesoderm induction. *Development* **137**, 1127-1135.
- Herrgen, L., Ares, S., Morelli, L. G., Schroter, C., Julicher, F. and Oates, A. C. (2010). Inter-cellular coupling regulates the period of the segmentation clock. *Curr. Biol.* **20**, 1244-1253.
- Honda, H., Motosugi, N., Nagai, T., Tanemura, M. and Hiiragi, T. (2008). Computer simulation of emerging asymmetry in the mouse blastocyst. *Development* **135**, 1407-1414.
- Huang, S. (2009). Non-genetic heterogeneity of cells in development: more than just noise. *Development* **136**, 3853-3862.
- Jaeger, J., Irons, D. and Monk, N. (2008). Regulative feedback in pattern formation: towards a general relativistic theory of positional information. *Development* **135**, 3175-3183.
- Kalmar, T., Lim, C., Hayward, P., Munoz-Descalzo, S., Nichols, J., Garcia-Ojalvo, J. and Martinez Arias, A. (2009). Regulated fluctuations in nanog expression mediate cell fate decisions in embryonic stem cells. *PLoS Biol.* **7**, e1000149.
- Kicheva, A., Pantazis, P., Bollenbach, T., Kalaidzidis, Y., Bittig, T., Julicher, F. and Gonzalez-Gaitan, M. (2007). Kinetics of morphogen gradient formation. *Science* **315**, 521-525.

- Kilfoil, M. L., Lasko, P. and Abouheif, E.** (2009). Stochastic variation: from single cells to superorganisms. *HFSP J.* **3**, 379-385.
- Kim, K. A., Spencer, S. L., Albeck, J. G., Burke, J. M., Sorger, P. K., Gaudet, S. and Kim do, H.** (2010). Systematic calibration of a cell signaling network model. *BMC Bioinformatics* **11**, 202.
- Klein, A. M., Nakagawa, T., Ichikawa, R., Yoshida, S. and Simons, B. D.** (2010). Mouse germ line stem cells undergo rapid and stochastic turnover. *Cell Stem Cell* **7**, 214-224.
- Kobayashi, T., Mizuno, H., Imayoshi, I., Furusawa, C., Shirahige, K. and Kageyama, R.** (2009). The cyclic gene *Hes1* contributes to diverse differentiation responses of embryonic stem cells. *Genes Dev.* **23**, 1870-1875.
- Lopez-Garcia, C., Klein, A. M., Simons, B. D. and Winton, D. J.** (2010). Intestinal stem cell replacement follows a pattern of neutral drift. *Science* **330**, 822-825.
- Losick, R. and Desplan, C.** (2008). Stochasticity and cell fate. *Science* **320**, 65-68.
- Mallavarapu, A., Thomson, M., Ullian, B. and Gunawardena, J.** (2009). Programming with models: modularity and abstraction provide powerful capabilities for systems biology. *J. R. Soc. Interface* **6**, 257-270.
- Masamizu, Y., Ohtsuka, T., Takashima, Y., Nagahara, H., Takenaka, Y., Yoshikawa, K., Okamura, H. and Kageyama, R.** (2006). Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. *Proc. Natl. Acad. Sci. USA* **103**, 1313-1318.
- Morelli, L. G., Ares, S., Herrgen, L., Schroter, C., Julicher, F. and Oates, A. C.** (2009). Delayed coupling theory of vertebrate segmentation. *HFSP J.* **3**, 55-66.
- Muramoto, T., Muller, I., Thomas, G., Melvin, A. and Chubb, J. R.** (2010). Methylation of H3K4 is required for inheritance of active transcriptional states. *Curr. Biol.* **20**, 397-406.
- Osawa, M., Hanada, K., Hamada, H. and Nakauchi, H.** (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273**, 242-245.
- Paszek, P., Jackson, D. A. and White, M. R.** (2010a). Oscillatory control of signalling molecules. *Curr. Opin. Genet. Dev.* **20**, 670-676.
- Paszek, P., Ryan, S., Ashall, L., Sillitoe, K., Harper, C. V., Spiller, D. G., Rand, D. A. and White, M. R.** (2010b). Population robustness arising from cellular heterogeneity. *Proc. Natl. Acad. Sci. USA* **107**, 11644-11649.
- Pezeron, G., Mourrain, P., Courty, S., Ghislain, J., Becker, T. S., Rosa, F. M. and David, N. B.** (2008). Live analysis of endodermal layer formation identifies random walk as a novel gastrulation movement. *Curr. Biol.* **18**, 276-281.
- Plusa, B., Piliszek, A., Frankenberg, S., Artus, J. and Hadjantonakis, A. K.** (2008). Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. *Development* **135**, 3081-3091.
- Polin, M., Tuval, I., Drescher, K., Gollub, J. P. and Goldstein, R. E.** (2009). *Chlamydomonas* swims with two 'gears' in a eukaryotic version of run-and-tumble locomotion. *Science* **325**, 487-490.
- Raj, A. and van Oudenaarden, A.** (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* **135**, 216-226.
- Raj, A., van den Bogaard, P., Rifkin, S. A., van Oudenaarden, A. and Tyagi, S.** (2008). Imaging individual mRNA molecules using multiple singly labeled probes. *Nat. Methods* **5**, 877-879.
- Raj, A., Rifkin, S. A., Andersen, E. and van Oudenaarden, A.** (2010). Variability in gene expression underlies incomplete penetrance. *Nature* **463**, 913-918.
- Ralston, A. and Rossant, J.** (2008). *Cdx2* acts downstream of cell polarization to cell-autonomously promote trophoblast fate in the early mouse embryo. *Dev. Biol.* **313**, 614-629.
- Rauzi, M., Lenne, P. F. and Lecuit, T.** (2010). Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature* (in press)
- Ribes, V. and Briscoe, J.** (2009). Establishing and interpreting graded Sonic Hedgehog signaling during vertebrate neural tube patterning: the role of negative feedback. *Cold Spring Harb. Perspect. Biol.* **1**, a002014.
- Riedel-Kruse, I. H., Muller, C. and Oates, A. C.** (2007). Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. *Science* **317**, 1911-1915.
- Rieger, M. A., Hoppe, P. S., Smejkal, B. M., Eitelhuber, A. C. and Schroeder, T.** (2009). Hematopoietic cytokines can instruct lineage choice. *Science* **325**, 217-218.
- Romaschoff, D. D.** (1925). Über die Variabilität in der Manifestierung eines erblichen Merkmales (*Abdomen abnormalis*) bei *Drosophila funebris* F. *Journal für Psychologie und Neurologie.* **31**, 323-325.
- Saka, Y., Hagemann, A. I., Piepenburg, O. and Smith, J. C.** (2007). Nuclear accumulation of Smad complexes occurs only after the midblastula transition in *Xenopus*. *Development* **134**, 4209-4218.
- Schroeder, T.** (2010). Hematopoietic stem cell heterogeneity: subtypes, not unpredictable behavior. *Cell Stem Cell* **6**, 203-207.
- Schroter, C. and Oates, A. C.** (2010). Segment number and axial identity in a segmentation clock period mutant. *Curr. Biol.* **20**, 1254-1258.
- Snippert, H. J., van der Flier, L. G., Sato, T., van Es, J. H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A. M., van Rheenen, J., Simons, B. D. et al.** (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134-144.
- Spencer, S. L., Gaudet, S., Albeck, J. G., Burke, J. M. and Sorger, P. K.** (2009). Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **459**, 428-432.
- Spudis, J. L. and Koshland, D. E., Jr** (1976). Non-genetic individuality: chance in the single cell. *Nature* **262**, 467-471.
- Stevenson, M., Muramoto, T., Muller, I. and Chubb, J. R.** (2010). Digital nature of the immediate-early transcriptional response. *Development* **137**, 579-584.
- Timoféeff-Ressovsky, N. W.** (1925). Über den Einfluss des Genotypus auf das phänotypen Auftreten eines einzelnen Gens. *Journal für Psychologie und Neurologie.* **31**, 305-310.
- Waddington, C. H.** (1942). Canalization of development and the inheritance of acquired characters. *Nature* **150**, 563-565.
- Wang, Z. and Jaenisch, R.** (2004). At most three ES cells contribute to the somatic lineages of chimeric mice and of mice produced by ES-tetraploid complementation. *Dev. Biol.* **275**, 192-201.
- Wartlick, O., Kicheva, A. and Gonzalez-Gaitan, M.** (2009). Morphogen gradient formation. *Cold Spring Harb. Perspect. Biol.* **1**, a001255.
- Wolpert, L.** (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1-47.
- Yamanaka, Y., Lanner, F. and Rossant, J.** (2010). FGF signal-dependent segregation of primitive endoderm and epiblast in the mouse blastocyst. *Development* **137**, 715-724.
- Yamashita, Y. M., Yuan, H., Cheng, J. and Hunt, A. J.** (2010). Polarity in stem cell division: asymmetric stem cell division in tissue homeostasis. *Cold Spring Harb. Perspect. Biol.* **2**, a001313.
- Ying, Q. L., Wray, J., Nichols, J., Battle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A.** (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519-523.