What’s all the noise about developmental stochasticity?

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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 Marinez-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 random 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; Timoféeff-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,
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 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 it behavior from microbial to metazoan within its life cycle is the slime mold *Dictyostelium*, which is studied by Johnathan Chub (University of Dundee, UK), who presented his group’s analysis of in vivo transcriptional dynamics by GFP tagging of the developmentally regulated *ecm4* gene (Stevens et al., 2010). Their approach allowed them to visualize nuclear foci of around 10-20 *ecm4* 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.
observed (Cohen et al., 2010a). Experimental analysis then revealed nearest neighbors, their model more closely fitted what they had incorporated signaling via the dynamic and intermittently forming basal protrusions that form between epithelial cells beyond their process or the final steady-state pattern. However, when they continuous in time, they could not reproduce either the refinement signaling via lateral inhibition that was both strictly near-neighbor and time. When they simulated this process computationally by modeling of bristle precursor cell specification in living embryos allowed them nearest-neighbor cells. Baum described how their time-lapse imaging arrangement invoke Delta-Notch-mediated lateral inhibition by Drosophila regular spacing of bristle precursor cells on the epithelium of the group’s studies of the role of stochastic signaling in determining the 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-κB 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-κB (Ashall et al., 2009). One such target is tumor necrosis factor (TNF), which is also the ligand that initiates signaling via NF-κB by releasing it from inhibitory I-κB subunits that anchor NF-κB in the cytoplasm, thus allowing its translocation to the nucleus. NF-κB also regulates I-κB and I-κBe transcription in delayed-negative-feedback loops that drive oscillations in NF-κB translocation by trapping NF-κB once again in the cytoplasm (Paszek et al., 2010a). However, if all cells in a tissue were to synchronize their NF-κB 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-κB and I-κBe-mediated negative-feedback loops show that they cause an increase in noise in the overall timing of the NF-κB 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 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 goosecoid 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 goosecoid domains (Harvey et al., 2010; Saka et al., 2007)?
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 Hiiiragi, 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 Hiiiragi (Max Planck Institute of Molecular Biomedicine, Münster, Germany) presented results of a Venus YFP promoter-trap screen designed to indentify 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 Hiiiragi 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 (Oshima 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 number of clones decreases while the size of the remaining cells that have made an earlier lineage choice to survive. To address whether cytokine signaling to hematopoietic progenitor cells causing a cell to follow a particular lineage, or selective, allowing cells 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).
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 flagellae 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 work, relating population heterogeneity and individuality, show a parallel to the pioneering analysis of stochasticity in

References

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