Research Networking Programmes

Science Meeting – Scientific Report

Scientific report (one single document in WORD or PDF file) should be submitted online within two months of the event. It should not exceed seven A4 pages.

***Proposal Title**: Complexity in Multicellular Environments*

Application Reference N°: 5026

1) Summary (up to one page)

How can we acquire a deep understanding of the most extraordinary of all non-equilibrium complex phenomena, i.e. life? While this broad question is still far from being answered, it is the inspiring and fundamental driving force that underlies this proposal. From a biological viewpoint, one soon realizes that to confront this question requires to transcend the molecular level and address it from the standpoint of a higher level of organization in which 'matter' can be qualified appropriately as 'living'. The minimal scale where life may be defined is the cell, while the higher forms of life require the collective organization of cells at different levels of complexity, from small multicellular organisms to the emergence of brain function. Cells and Tissues are thus the two fundamental levels in which progress may be most operative. In this context, different yet complementary approaches, that soon reveal the appearance of different scales, are possible. First, one may try to understand how the mechanical properties and the inner functioning of the cell emerge from the collective dynamics of molecular motors, and their interplay with biochemical regulation. Second, one may try to understand the communication mechanisms between cells and their collective organization. While in the recent years a lot of progress has been achieved by using the former approach, the latter viewpoint has been successfully addressed by a combination of experimental and modeling tools only very recently. Multicellular environments have been usually studied by biologists at the genetic level, trying to identify the genes that promote interactions between cells. More recently, a complementary approach addressing the various "physical parameters" involved in the growth and the morphogenesis of a tissue has been developed using approaches from Statistical Mechanics and Complex Systems. In addition, also at the tissue level different scales at which tissues grow can be studied. At the cell scale one can study the local rearrangements of cells while at larger scales it is also possible a hydrodynamic framework. For example, recent

experiments seem to show that in many cases the tissue can be considered as a liquid over long time scales of the order of the cell division times. All in all, multicellular environments illustrate how coherent complex behavior develops at the collective level from the hierarchical interaction of individual units and scales that ultimately determine the biological function

2) Description of the scientific content of and discussions at the event (up to four pages)

In the first talk, by James Sharpe, multicellular embryonic development was presented as a complex system composed by a "network of networks". This point of view, rooted on the speakers' previous work, together with attention to active cell behaviour and tissue movements, was applied to the study of limb bud development. The goal was to build a data driven model of limb morphogenesis. For this, careful observation and image analysis of the developing limb bud was used to create computer models of the evolution of shape and size of the limb bud. These models are used as the "playground" on top of which molecular interactions of the genes involved in the process can be modelled and simulated. By separating these two levels of the process, it is possible to study the molecular aspects in a realistic setting without the need to build a working model for the temporal evolution of the limb bud's shape. Instead, the idea is to reproduce the limb's skeletal patterning. Models based solely on the idea of morphogen gradients are shown to be at odds with the phenotypes of mutants of the several genes candidates to be such morphogens. In turn, purely reaction-diffusion processes following the ideas of Alan Turing also do not seem enough to provide an explanation to the phenomena observed. However, when self-organized Turing patterns are combined with positional information supplied by gradients of FGF and Hox genes, realistic patterns of finger formation are obtained. This talk introduced a subject that would be recurring during the whole workshop: genetic networks, interactions between them, and how they can be modelled in a data driven approach. As all talks in the workshop, this one was followed by a lively discussion. Actually, although speakers were encouraged to keep their talks not longer than 30 minutes in order to have 10 minutes for discussion, discussions were so lively and interesting that systematically they took longer until they had to be interrupted, making all sessions of the workshop go over the scheduled time.

The talk by David Míguez continued the general topic of gene regulation in embryonic development. He presented his work on the genes Smad2 and Smad3, members of the transforming growth factor beta (TGF-β) pathway that plays key roles in development and cancer. The work presented studied the development of the chick's neural tube, where Smad2 and Smad3 seem some times to have antagonist and sometimes cooperative roles. By using clever *in vivo* and *in silico* experiments, Dr. Míguez showed that different multimers formed by these genes can have different roles, explaining the apparently paradoxical experimental results.

Marta Ibañes continued presenting work with a strong developmental biology flavour. Based on theoretical and mathematical tools, she discussed the simultaneous interactions of Notch receptors on the cell membrane with several ligands. She presented two distinct cases: when two different ligands are competing to bind the receptor, and the case in which a ligand can bind in *trans* (that is, a ligand from one cell activates a receptor on a neighbour cell) or *cis* (ligands from a cell can activate receptors from the same cell). Through

mathematical analysis of models for these cases, the conclusion is reached that competition for signalling can appear in two ways: through actual competition, but also when the competing factors play apparently the same role but have different competing strengths.

In the next session, Xavier Trepat presented work on expansion of sheets of cells, a topic relevant for development and wound healing. His main question concerns the mechanism driving the movement of these sheets. Is it leading cells dragging the sheet forward? Is each individual cell mechanically self-propelled? Does cell proliferation push the sheet forward? Measuring forces exerted by cells on their substrate, it was determined that the role of cell proliferation is small, and that forces appear many rows of cells behind the leading cell, showing that there is more to this collective cell movement than drag by leading cells. Next the problem of the closure of a circular gap was presented, where two mechanisms where though to possibly contribute: cell crawling and the contraction of an actomyosin ring formed in the cells bordering the gap. Experimental work together with mathematical modelling showed that actually both mechanism play a role, since models based on each of them alone cannot fit experimental results, while a combination of them does.

The talk of Christian Schröter dealt with the question of how complex multicellular organisms arise from the zygote by cell divisions and fate specialization. The mouse pre-implantation embryo contains three distinct cell lineages. While the differentiation between trophoblast and the inner cell mass is driven purely by positional cues (cells in the exterior or interior part of the embryo), the differentiation of the inner cell mass into primitive endoderm and epiblast is controlled by a complex regulatory network, where nanog, FGF and Gata factors play important roles. As a model system to study the more general topic of fate specialization, Dr. Schröter used mouse embryonic stem cells to investigate how the lineages of primitive endoderm and epiblast are reliable populated from a small number of cells. Cell cultures of these stem cells offer a suitable model system to address the rules underlying the primitive endoderm and epiblast cell specification, and ongoing work on this regulation was presented. During the discussion following this talk, Jose María Frade asked whether epigenetics may be playing a role in this cell decision process, raising an interesting series of thoughts on the topic.

Closing the morning sessions, Nick Monk continued discussing cell fate decisions, but from a purely mathematical perspective. Lateral inhibition mediated by the Notch pathway was an important topic. He showed how in bifurcation theory of the dynamics of differentiation mediated by Delta and Notch, cells go first to a dynamical state termed a saddle, and from there choose a fate. Considering the time scales of the molecular interactions between ligands and receptors through the inclusion of time delays in the models can give rise to oscillations in cellular states that can be sustained or transient before choosing a fate and differentiating. Transient oscillations offer a mechanism through which cells can undergo changes between different fates before a definitive fate decision without need of extrinsic factors to stop the oscillations. To translate these ideas into more realistic settings of intercellular interactions, a cellular Potts model was presented that can take account of cell movement, growth and division, on top of witch the Delta-Notch dynamics can be simulated.

Javier Muñoz-García presented more work on pattern formation, but featuring a different system: the formation of a pattern of nitrogen fixing cells, termed heterocysts, in filaments of cyanobacteria of the genus *Anabaena*. Filamentous cyanobacteria are a model organism for the transition between unicellular and multicellular living forms, and heterocyst differentiation is a primary example of cell differentiation in simple organisms. Mathematical modelling of the dynamics of the genes *hetR*, *patS* and *hetN*, together with filament growth through cell differentiation, lead to a quantitative understanding of experimental results in this field.

Ekkehard Ullner presented mathematical models of how noisy synthetic genetic oscillators in bacteria can couple through the production and diffusion of quorum sensing molecules. These processes can lead to very rich dynamical behaviours, including synchronization and chaos. Interestingly, analogies to neuronal differentiation in the models were pointed out, making direct reference to the work presented earlier by Nick Monk.

Tobias Bollenbach presented what in principle seemed an off-topic: how bacteria respond to drug combinations in situations where two signals tell the cell to do opposing things. In particular, he presented the case of antibiotic combinations that lead to gene regulatory conflicts. His presentation had an important methodological aspect, showing how to use principle component analysis to identify the most important trends in experimental results. His talk ended introducing his ongoing research, in which he referred to topics already discussed during the day. Using methods similar to his work in bacteria, he is investigating how combinations of signals are integrated in the development of the neural tube in vertebrates.

The last session started with the talk of Enrique Martín Blanco. The work presented was related to Xavier Trepat's talk, but now collective cell migration was studied *in vivo* using zebrafish epiboly as a model system. The experimental study was complemented using a fluid-like model for tissue flow, including a description based on Stokes' equation. The main question behind the study is how to make an organism starting from a small number of cells, and focusing on the role collective cell migration plays in this process.

Johannes Jaeger tried to reverse engineer the evolution of developmental systems. For this he studied and compared the role of gap genes, pair rule genes and segment polarity genes in the embryonic segmentation process of different species of flies. Most of these species are only distantly related, and the common task of segmenting the body axis is achieved using different regulatory relations between the studied set of genes.

Finally, the last talk was a contributed one by Pau Formosa. Continuing with the topic of biological pattern formation, he addressed these processes in plants. He described mathematical models, conceived in collaboration with experimentalists, on how auxin influx carriers affect vascular bundle formation in the plant *Arabidopsis thaliana*. His talk was a nice example showing how recurring phenomena that had appeared in different talks during the day play a role in the formation of patterns in very different biological systems.

3) Assessment of the results and impact of the event on the future directions of the field (up to two pages)

The workshop was a complete success. Several meetings and discussions among participants considering possible future collaborations took place. For instance, Marta Ibañes, Nick Monk, Jose María Frade and Saúl Ares got together after the meeting to discuss how results and methods presented in the talks could be used to model neurogenesis of stratified neuroepithelia. Moreover, several topics appeared in different talks, showcasing their importance for different problems and their transversal applications. For instance, image analysis of whole tissues appeared playing different roles in the talks James Sharpe, Xavier Trepat and Enrique Martín Blanco. Genetic networks was a recurring topic mentioned by James Sharpe, David Míguez, Marta Ibañes, Christian Schröter, Nick Monk, Javier Muñoz-García, Ekkehard Ullner and Johannes Jaeger. Actually, genetic networks with strikingly similar topologies appeared in different contexts in the presentations of Christian Schröter, Nick Monk, Johannes Jaeger and Tobias Bollenbach. The increasing importance of post-transcritional regulation through the formation of protein multimers appeared in the talks of David Míguez and Javier Muñoz-García, and it is also related to past work of Christian Schröter. How biological system integrate conflictive signals was an issue in the work presented by Marta Ibañes, David Míguez and Tobias Bollenbach.

Besides particular issues, the main underlying theme was the importance of data driven mathematical and computational modelling. The union of experiment and modelling showed to be more than the sum of their individual aspects, allowing for deeper insights into complex biological phenomena.

4) Annexes 4a) and 4b): Programme of the meeting and full list of speakers and participants

Annex 4a: Programme of the meeting

Session 1: **9:00 - 9:40 James Sharpe: Multiple levels of feedback control during organogenesis**
9:40 - 10:20 David Míguez: Smad2 and Smad3 cooperate and antagonize in vertebrate neurogenesis
10:20 - 11:00 Marta Ibañes: Competition in signaling dynamics facilitates patterning during animal embryonic development

Coffee break

Session 2: **11:30 - 12:10 Xavier Trepat: Forces and waves during epithelial growth**
12:10 - 12:50 Christian Schröter: Integration of transcription factor and signalling activity in a developmental lineage decision switch
12:50 - 13:10 Nick Monk: Modelling cell fate choices in fluctuating environments

Lunch

Session 3: **14:30 - 15:10 Javier Muñoz-García: Formation and maintenance of nitrogen fixing cell patterns in filamentous cyanobacteria**
15:10 - 15:50 Ekkehard Ullner: Multi-stability and hopping of the noisy repressilator with quorum sensing
15:50 - 16:10 Tobias Bollenbach: Bacterial responses to drug combinations

Coffee Break

Session 4: **17:00 - 17:40 Enrique Martín Blanco: Complexity (or simplicity) on growth and form during development**
17:40 - 18:20 Johannes Jaeger: Life's Attractors: the Evolutionary and Developmental Dynamics of the Gap Gene Network
18:20 - 18:40 Pau Formosa-Jordan: Auxin influx carriers can control periodic patterning in plants

Annex 4b: Full list of speakers and participants

James Sharpe, Centre de Regulació Genòmica, Barcelona
David Míguez, Universidad Autónoma de Madrid
Marta Ibañes, University of Barcelona
Xavier Trepat, Institut de Bioenginyeria de Catalunya (IBEC)
Christian Schröter, Department of Genetics, University of Cambridge
Nick Monk: University of Sheffield
Javier Muñoz-García, Universidad Carlos III de Madrid
Ekkehard Ullner, University of Aberdeen
Tobias Bollenbach, IST Austria
Enrique Martín Blanco, Molecular Biology Institute of Barcelona
Johannes Jaeger, Centre de Regulació Genòmica, Barcelona
Pau Formosa-Jordan, University of Barcelona
José María Frade, Cajal Institute – CSIC
Saúl Ares, Spanish National Biotechnology Centre (CNB) – CSIC
Javier Buceta, Computer Simulation and Modeling Lab, Parc Científic Barcelona

Luis Morelli, from the University of Buenos Aires participated in the organizing committee, but could not assist to the actual workshop.

Many more participants of the European Conference on Complex Systems ECCS'13 that had not registered for the Complexity in Multicellular Environments meeting assisted as audience and took part in the discussions. The room assigned to our meeting by the ECCS'13 organization was full most of the sessions.