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Spatiotemporal control of cellular microenvironments with photonics

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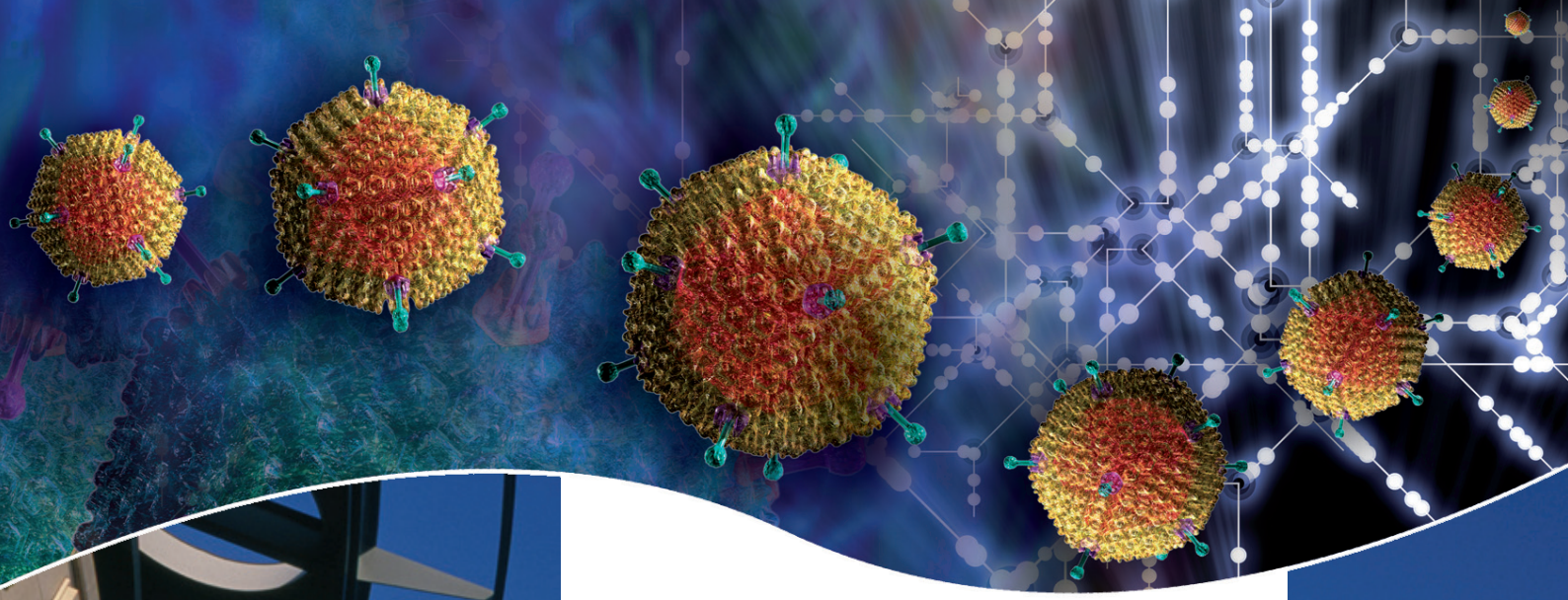

SGM Spring 2010 Meeting

Edinburgh International Conference Centre

29 March–1 April 2010

Systems, Mechanisms and Micro-organisms

ABSTRACTS

DELIVERING MODERN MICROBIAL SCIENCE

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Stochastic computer simulations of the molecular interaction networks can be used to understand molecular events leading to the emergence of population heterogeneity and generate the hypotheses leading experimental work. I will present the stochastic kinetic model of the two component system signalling (TCS) showing different modes of phenotypic switching in this signal transduction network. The TCSs are involved in the regulation of adaptive responses to environmental cues and host-pathogen interaction. Therefore, the stochastic effects in the TCSs may potentially lead to the emergence of many phenotypic switching phenomena observed in the bacterial populations. I will also present computer simulations demonstrating propagation of the gene expression and signalling noise to the level of metabolic networks and discuss the consequences of these stochastic events for the understanding of the epigenetic changes in global physiological state of the bacterial cell.

Differential control of bacteria gene expression

Eduardo A. Groisman

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Abstract not received

Individuality of bacterial responses to antimicrobials

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Bacteria behave as individuals. Mutation and genetic exchange are important drivers of bacterial individuation, but these events are rare. At higher frequencies, genetically identical cells display metastable variation in growth rate, response kinetics, stress resistance, and other quantifiable phenotypes. These between-cell differences arise from non-genetic sources, such as stochastic gene expression and asymmetric partitioning of components at cell division. Phenotypic variation at the single-cell level generates phenotypic diversity at the population level. This diversity is critical for bacterial persistence in fluctuating environments. It ensures that some individuals will survive a potentially lethal stress that would otherwise extinguish the population. For example, the refractoriness of bacterial infections to antibiotic therapy has been ascribed to spontaneous variants ('persisters') that survive and regrow despite prolonged antibiotic exposure. The persisters are not antibiotic resistant mutants and it is unclear why they tolerate antibiotics that kill their genetically identical siblings. Our studies focus on the mechanistic basis of the reversible persister switch in the clinically important micro-organism *Mycobacterium tuberculosis*. We use automated time-lapse microscopy, microfluidics, and microelectromechanical systems to analyse bacterial responses to antibiotics at the single-cell level. Our studies show that the conventional interpretation of the persister phenomenon – viz., that persistence is due to pre-existing subpopulations of dormant cells – is incorrect. Instead, we find no correlation between the growth rates of individual cells and their probability of survival during antibiotic exposure. We also find that the survival probability of closely related sister cells is strongly and positively correlated. These observations suggest that the (unknown) factors that determine whether a cell lives or dies are metastable within individual cell lineages.

O_2 be a pathogen: signalling of host microenvironments to promote *Shigella* virulence

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EUKARYOTIC CELL BIOLOGY

Spatiotemporal control of cellular microenvironments with photonics

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Living cells respond to biochemical and mechanical stimuli. A quantitative understanding of the interplay between signaling and cell mechanics requires experimental methods to stimulate cells flexibly with biochemical and mechanical cues. We use optical tweezers in combination with functionalized microparticles to control the mechanical and biochemical microenvironment of individual cells. Our research focuses on investigating phagocytosis and chemotaxis in single immune cells. I will discuss the mechanics of macrophages and bacterial pathogens during the early stages of phagocytic uptake. Our studies revealed that macrophage filopodia act as tentacles which pull bound objects with discrete steps and a load-dependent velocity towards the cell for further phagocytosis. In addition, I will present a novel method for flexible biochemical cell stimulation. This method is based on optically manipulated microsources, which

steadily release molecules. The technique enables control over biochemical cellular microenvironments down to length scales of one micrometer and timescales from an hour down to seconds. We demonstrated this technique by guiding and perturbing the migration of single human neutrophils.

Towards systems analysis of small GTPase activities in early HIV infection

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Viruses are obligatory cellular parasites, with their replication crucially depending on host cell functions. They have evolved strategies to modulate host cell cytoskeleton dynamics and activate signal transduction pathways to facilitate entry into new target cells and release of progeny virus from infected cells. Therefore viruses are ideal tools to study signalling and cytoskeleton networks in an integrative way by comparing the dynamics of such virus exploited networks with the dynamics induced by natural ligand interaction. Changes in cytoskeletal structure are tightly regulated by the small GTPases like RhoA, Rac1 and Cdc42.

Using dominant-negative and constitutive active forms of those GTPases we identified RhoA and Rac1 as factors contributing to changes in microtubule stability and reduced susceptibility to virus infection, thus identifying microtubules as potential cellular target for virus-cell fusion (Malinowsky *et al.*, 2008). In order to visualize and quantify changes in signalling we have recently established a FRET based assay to detect activation of RhoA, Rac1 and Cdc42 using Raichu-FRET probes described by M Matsuda's group in 2003 (Yoshizaki *et al.*, 2003).

The G_β protein Gpb1 and the co-repressor TupA bind to Protein Kinase A Tpk2 to act as antagonistic molecular switches of fungal morphological changes

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Paracoccidioides brasiliensis is one of a group of six phylogenetically related ascomycete fungi that are adapted for survival in mammalian hosts and which cause millions of infections every year. In *P. brasiliensis* the transition from the mycelium to the pathogenic yeast form of is controlled by the cAMP-signalling pathway and during the transition there is a change in the expression of the G_β-protein Gpb1 that interacts with adenylate cyclase. We have exploited the fact that the cAMP-signaling-pathway of *S. cerevisiae* does not include a G_β-protein, so as to use this organism to probe the functional role of Gpb1. We present data that indicates that Gpb1 and the transcriptional regulator TupA both bind to the PKA protein Tpk2; through which they act as antagonistic molecular switches of cell morphology, with TupA and Gpb1 inducing and repressing filamentous growth, respectively. In *S. cerevisiae*, PbTupA-induced filamentation occurs at two levels, with the early production of numerous pseudohyphae, a hyperfilamentious state, followed by the formation of an invasive projection and aerial stalk. In contrast, overexpressing ScTup1 represses the filamentous growth of *S. cerevisiae*, suggesting that an organisms Tup protein determines the morphological outcome. Conversely, Gpb1, which binds to PbTpk2, but not ScTpk2, could over-ride the effect of PbTupA to repress filamentous growth in *S. cerevisiae*. Our findings define a mechanism for controlling the morphological switch that underpins the virulence of dimorphic fungi.

The responses of fungal pathogens to environmental insults

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The success of *Candida albicans* as a human pathogen is enhanced by a battery of fungal virulence factors (such as cellular morphogenesis, adhesins and secreted hydrolytic enzymes) and fitness attributes (such as stress responses and metabolic adaptation). The stress responses help *C. albicans* combat host immune defences, which attack the pathogen with a battery of environmental insults that include reactive oxygen and nitrogen species. These stress responses appear to be regulated spatially and temporally during disease establishment and progression. We (and others) have begun to dissect the molecular mechanisms by which *C. albicans* responds to these oxidative and nitrosative stresses. Our approach is to combine mathematical modelling with molecular biology and genomics with a view to generating models that help us to predict the behaviour of *C. albicans* within the relatively complex and varied microenvironments of the host.