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ITMO Biologie Cellulaire
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JM Dura & T Galli for ITMO BCDE

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Current challenges in cell-cell communication

July, 1st 2016,

Auditorium Biopark

Paris, France

Confirmed speakers/orateurs

Maria Cecilia ANGULO , Paris, France

Gerard KARSENTY, New York, USA

Danijela MATIC VIGNJEVIC, Paris, France

Simona PARRINELLO, London, UK

Graca RAPOSO, Paris, France

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Pre-Program

9 :15 – 9 :50	Welcome
9 :50 – 10 :00	Introduction by Corinne ALBIGES-RIZO, IAB, Grenoble, France
10 :00 – 10 :45	Gérard KARSENTY, Columbia University, New York, USA The impact of bone on whole body physiology
10 :45 – 11 :30	Graça RAPOSO, UMR144, Institut Curie, Paris, France Extracellular Vesicles in intercellular communication, from neurodegeneration to pigmentation
11 :30 – 12 :00	Coffee break
12 :00 - 12 :45	Danijela MATIC VIGNJEVIC, UMR144, Institut Curie, Paris, France Carcinoma-associated fibroblasts induce protease-independent cancer cell invasion
12 :45 – 2 :30pm	Lunch and Poster session
2 :30 – 3 :30pm	Short talks session (selected from poster abstracts)
3 :30 – 4 :15pm	Maria Cecilia ANGULO, UMR8154, Univ. Paris Descartes, Paris, France Synapses between interneurons and oligodendrocyte progenitors in the developing neocortex
4 :15 – 5 :00pm	Simona PARRINELLO, MRC, Londres, UK Cell-cell communication in the normal and malignant neurovascular niche
5 :00 – 5 :10pm	Concluding remarks

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Abstract List

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BIZZOTTO Sara	sara.bizzotto@inserm.fr	Apical progenitor spindle length, soma size and number influence the developing cerebral cortex
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VALENZUELA José I	jose-ignacio.valenzuela@curie.fr	Intra- and intercellular trafficking of ephrins: the filopodia connection

Circulating tumor cells adhere to vascular fibronectin deposits via focal adhesions during liver metastasis

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Keywords: Fibronectin, Focal Adhesions, Circulating Tumor Cells, Extravasation, Liver metastasis

The interaction between circulating tumor cells (CTCs) and endothelial cells during extravasationis critical for metastatic colonization, but its mechanisms remain elusive. Here we report that the luminal side of liver blood vessels contains fibronectin plaques that occur more frequently in mice bearing primary tumors. We show in vivo that cancer cells attach to fibronectin plaques using focal adhesions. Disruption of focal adhesion formation in cancer cells by talin1 knockdown reduces their attachment to endothelial monolayers and ability to undergo transendothelial migration and also reduces liver metastasis formation in vivo. Consistently, talin1 expression levels in patients' CTCs correlates with prognosis and therapy response. Together, these findings uncover a new mechanism for liver metastasis formation involving an active contribution of hepatic vascular fibronectin and focal adhesions in cancer cells.

*Data currently under revision

INTERPOL: INTERaction and POLarisation of human hematopoietic stem cells in artificial niches

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Keywords: HSCs, Bone marrow niche, polarization, cytoskeleton, differenciation

For a time now we have tried to amplify *in vitro* hematopoietic stem cells (HSCs) for their therapeutic use in bone marrow engraftment. The main problem faced at the moment is maintaining the stemness of HSCs during the process. Studies have shown that the cellular environment (niche) of HSCs in the bone marrow plays a critical role in cueing asymmetric division, and thus in controlling self-renewal, quiescence and differentiation of HSCs. Notably, it has been demonstrated that mesenchymal stromal cells (MSCs) are essential for maintaining HSCs, and are now frequently used as feeder cells *in vitro.*

In this project, we are aiming at understanding the roles of the different cellular component of the niche in the maintenance or differentiation of HSCs. We will assess the effect of cell contact on its polarisation and on asymmetric division

The experimental set up will be polymer-built micro-wells that will represent a physical constraint allowing us to put in contact single cells from two different cell types, typically a HSC and a cell of the hematopoietic niche. The organisation and polarisation of the cytoskeleton will help us observe asymmetric division and eventual cell/cell interaction.

Apical progenitor spindle length, soma size and number influence the developing cerebral cortex

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The ventricular zone of the developing cerebral cortex is a pseudostratified epithelium that contains progenitors undergoing precisely regulated divisions at its most apical side. Perturbations of apical progenitors often lead to malformations of cortical development. We questioned unexplored characteristics regarding the most apical side of the ventricular zone focusing on the mitotic process. Mitotic spindle assembly and function influence progenitor divisions. The interplay between metaphase spindle length and cell size is believed to influence chromosome segregation and establishment of the division plane. We discovered that progenitor metaphase spindle length diminishes from early- to mid-corticogenesis, despite average cell size remaining the same. We further questioned whether cell density influences metaphase cell size, showing that this was the case but only at early-corticogenesis. Diminished sizes were hence observed at higher densities. More apical mitoses were also observed at this stage compared to mid-corticogenesis. We also show how these characteristics are perturbed in Eml1 mutant mice which exhibit a proportion of dividing progenitors outside the ventricular zone associated with a malformation known as subcortical band heterotopia. Increased spindle length identified at early corticogenesis in mutant brains may lead to progenitor delamination, since cells are also larger than control cells.

This work underlines how mitotic spindle length changes during cortical development and its mutual interactions with the tissue environment, likely to have an influence on the position of neural progenitors.

Modulation of calcium responses and cell death during *Shigella* invasion of epithelial cells

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Keywords: Calcium signaling, Host-pathogen interaction, Type III effector, IpgD, Calpain, Talin

Shigella, the agent of bacillary dysentery, invades colonic epithelial cells where it disseminates causing an intense inflammation and tissue destruction. To efficiently promote infection, *Shigella* injects virulence effectors via a type III secretion system (T3SS) into host cell to divert processes involved in cytoskeletal rearrangements and processes regulating inflammatory signals or tissue integrity. Among the *Shigella* T3SS effectors, IpgD acts as a phosphatidylinositol (PI) (4, 5) bisphosphate phosphatase, which dephosphorylates PI (4,5) P2 to generate PI(5)P. IpgD-mediated hydrolysis of PI (4, 5) P2 favors actin polymerization during cell invasion and negatively regulates the migration of T cells. PI(5)P produced by IpgD was also shown to up-regulate the PI3K/AKT cell survival pathway and recycling of the Epidermal Growth Factor receptor. Here, we show that IpgD is responsible for a decrease in the recruitment of inositol-triphosphate (InsP3) receptors at invasion sites. Ca2+-imaging experiments indicate that during the early stages of bacterial invasion, IpgD favors the elicitation of local Ca2+responses at *Shigella* invasion sites, and limits the induction of global Ca2+ responses. Fluorescence Recovery After Photobleaching (FRAP) experiments indicate that actin foci induced by the *ipgD* mutant show faster diffusion kinetics than those in foci induced by WT. Modeling studies support the notion that the local decrease in InsP3 levels and of its diffusion kinetics triggered by IpgD at entry sites accounted for the modulation of local to global Ca2+ responses during *Shigella* invasion. We show that over prolonged infection kinetics, IpgD inhibits InsP3-dependent global Ca2+ responses induced by *Shigella* or agonists. IpgD-mediated inhibition of Ca2+ signals delays the activation of calpain, the degradation of the focal adhesion protein talin, and cell death during bacterial intracellular replication. Our results provide evidence that IpgD is a critical T3SS effector of *Shigella* regulating the transition from local to global Ca2+ signals, and preserving cells from death linked to loss of adhesion.

A potential role for Weibel-Palade Body exocytosis in cell-cell communication between endothelial cells and pericytes during angiogenesis

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Endothelial cell (EC) activation is a cellular process by which ECs – quiescent in mature vessels – are activated under various environmental stimuli such as VEGF, histamine, thrombin or sheer stress. EC activation occurs in vascular homeostasis during healing/reparation or inflammation events, and leads to EC loss of quiescence and Weibel-Palade Body (WPB) exocytosis. WPBs are endothelial-specific storage organelles whose primary constituent is von Willebrand Factor (vWF). WPBs also contain pro-inflammatory molecules (P-selectin, Interleukin-8) and pro-angiogenic factors such as Angiopoietin-2 (Ang2), a ligand for Tie-2 involved in tip cell migration and endothelium destabilization. EC activation is also the first step of angiogenesis, a process allowing new vessel development from pre-existing ones. However, if the role of WPBs is well studied in contexts of vascular homeostasis, little is known about the role of WPBs in angiogenesis. We aimed to characterize the role of WPB exocytosis during angiogenesis by using mouse retina model.

In this model, we observed that the density of WPBs in the developing vessel network of P6 mice was heterogeneous: vessels close to the vascular front – that are actively angiogenic and exposed to higher levels of VEGF – contained less WPBs than vessels close to the optic nerve. Targeting VEGF signaling pathway with a chimeric antibody directed against VEGF (anti-VEGF) significantly reduced VEGF-induced WPB exocytosis in ECs, and intravitreal injection of VEGF increased the density of WPBs in remodeling vessels of developing retina. These results suggested that WPB exocytosis participated in the remodeling process of the vascular network.

We next analyzed the localization of Ang2, and observed that Ang2 colocalized with vWF in ECs of the retina vascular network, suggesting that Ang2 is contained in WPBs of the retina vessels. Ang2 is known to regulate pericyte recruitment to vasculature and we showed *in vitro* that Ang2 silencing in ECs was sufficient alone to improve pericyte migration. Similarly to WPB distribution, vessels close to the optic nerve were more covered by pericytes than vessels close to the vascular front, suggesting a functional correlation between WPB exocytosis and pericyte recruitment during retina angiogenesis. Therefore we studied pericyte coverage after intravitreal injection of anti-VEGF. Inhibition of WPB exocytosis by anti-VEGF increased pericyte density on vessels close to the vascular front but had no effect on vessels close to the optic nerve.

To conclude, we propose a model in which cell-cell communication between endothelial cells and pericytes is mediated by WPB exocytosis that differentially controls pericyte recruitment to the different regions of developing retina. Vessels close to the vascular front are exposed to high levels of VEGF that induce WPB exocytosis and Ang2 secretion. The presence of Ang2 inhibits pericyte recruitment to vasculature. On the contrary, vessels close to the optic nerve are less exposed to VEGF. WPBs remain stored in ECs and the absence of Ang2 release enables pericyte recruitment to newly formed vessels.

Caveolae function at melanocyte-keratinocyte contact sites during pigmentation

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Keywords: Pigmentation; caveolae; caveolin-1; melanocytes; melanosomes; keratinocytes

Skin pigmentation relies on the cooperation of two cell types, melanocytes and keratinocytes. While melanocytes form specialized organelles in which melanin pigment is synthesized and stored, this pigment is further transferred to neighboring keratinocytes to photoprotect the organism. Different cellular mechanisms supporting the melanin transfer have been proposed but little is known about the molecular mechanisms and players involved. However, specific features might be required at the melanocyte-keratinocyte interface to warrant the melanin transfer.

Ultrastructural analyses of such interfaces in different samples (human skin biopsies, synthetic epidermis, primary melanocytes and keratinocytes co-cultures) reveal an accumulation and polarization of caveolae. Caveolae are plasma membrane invaginated structures which contribute to lipid homeostasis, signaling transduction, mechanosensing or endocytosis. Impairing caveolae formation and function by knocking down caveolin-1 in melanocytes decreased the amount of transferred pigment in keratinocytes and concomitantly increased the melanin content in melanocytes. Consistently, caveolin-1-depleted melanocytes harbored an overall increase in the number and pigmentation status of melanosomes as well as expression levels of key melanosomal components. Alternatively, activating melanocytes using solar UV radiations positively modulated the expressions of all caveolae components.

Altogether, our data propose that melanocytes modulate the formation and function of caveolae to further control the production and transfer of pigment from melanocytes to keratinocytes. We are currently characterizing by which pathways (signaling and/ or mechanosensing) caveolae control skin pigmentation.

Structural and functional analysis of tunneling nanotubes (TnTs) in PC12 cells

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Tunneling nanoTubes (TnTs) are thin plasma membrane bridges mediating transfers of materials and signals between cells. Due to large disparity of TnT-like structures in neuronal, immune, cancer or epithelial cells, high and super resolution approaches are necessary for full characterization of these yet poorly understood routes of cell-to-cell communication. We propose here imaging strategies designed to dissect structural and dynamic aspects of TnT formation and function in fixed or living PC12 cells. Through time-*g*ated Continuous Wave STimulated Emission Depletion (*g*CW STED) nanoscopy associated to deconvolution, we provided nanoscale details of membrane and cytoskeleton organizations in two subtypes of tunneling nanotubes, namely TnT1 and TnT2. In fixed PC12 cells, TnT1 (length, several tens of µm; diameter, 100-650 nm) exhibited a large trumpet-shaped origin, a clear cytosolic tunnel and different bud-shaped connections from closed-ended to open-ended tips. TnT1 contained both actin and tubulin. TnT2 (length, max 20 µm, diameter, 70-200 nm) only contained actin without clear cytosolic tunnel. In living PC12 cells, we observed through *g*CW STED additional details, unrevealed so far, including a filament spindle emerging from an organizing center at the origin of TnT1 and branched or bulbous attachments of TnT2. We were also able to monitor dynamics of bud-shaped tips and intercellular transfer of WGA-labeled cellular elements through time-gated confocal microscopy. Our work identified new structural characteristics of two subtypes of TnTs in PC12 cells as well as dynamics of formation and transfer through complementary imaging methods combined with image processing. Therefore, we could achieve maximum lateral resolution and sample preservation during acquisitions to reveal new insights in TnT studies.

Molecular and cellular characterization of the dormant and injury-activated mouse and human spinal cord stem cell niches

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Keywords: neural stem cells ; niche ; cell fate ; spinal cord injury

The adult spinal cord contains a pool of neural stem cells in a niche situated around the central canal. These cells, present in rodents and in man, are able to form new neuronal and glial cells in vitro. In contrast to the brain, the spinal cord niche is in a dormant state and produces no or few new cells in the normal situation. The niche can be readily activated and generate new cells in spinal cord traumas. Our lab has characterized the human and mouse spinal cord niches in depth and showed that far from being a single layer of cells, the central canal region is composed of several cell types which show immaturity features and that the niche maintains the activation of several developmental signalling pathways.

We explored the molecular mechanisms underlying niche activation after injury. We used laser microdissection and microarray screening to decipher the early molecular events taking place during activation. This led us to show that the Mapk/Ras signalling is rapidly activated in the niche after injury and we identified several potential targets of this pathway which may orchestrate the activation. In order to describe further the spatial organisation of the niche we microdissected the ventral, lateral and dorsal parts of the central canal. This will allow us to identify genes which are more specific of the different regions of the niche. To explore the conservation of the niche in mammals, the human spinal cord niche is also explored by immunohistochemistry and transcriptome analysis

Molecular control of radial intercalation in a vertebrate epithelium.

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Keywords: Xenopus; Epithelia; Multiciliated cells; Cell movements; Radial intercalation; Actin cytoskeleton; Tyrosine kinase receptors; c-KIT; SCF

The embryonic epidermis of the amphibian *Xenopus laevis* is a mucociliary epithelium consisting of mucus-secreting cells, ionocytes, serotonin-secreting cells and multiciliated cells (MCCs). During development, the MCCs, SSCs and ionocytes are born within the inner epithelial layer, then migrate to the outer layer, where they intercalate among the mucus-secreting cells. The precursors of the MCCs emerge at the surface of the outer layer as indidual cells and only in correspondence of vertexes (points of contact among three outer layer cells). We show here that the signalling pathway dependent on the transmembrane tyrosine kinase receptor c-KIT and its cognate ligand SCF is required for the proper intercalation of the MCCs. SCF is expressed by the cells of the epithelial outer layer, while expression of c-KIT is restricted to MCCs. The disruption of the SCF/c-KIT pathway results in severe abnormalities in the distribution of MCCs which mostly fail to intercalate within the outer epidermal layer. We also show that, upon SCF/c-KIT pathway inhibition, the MCCs spend more time wandering between the inner and outer layer, and hesitate among several vertexes. We propose a model in which the SCF/c-KIT pathway is required to polarize the motility of intercalating MCCs towards the outer layer and to anchor intercalating MCCs to outer layer vertexes.

Role of mechanical tensile forces in Notch signaling pathway

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During the development and throughout embryogenesis and adult life, epithelial tissues must grow and/or regenerate to maintain their integrity. In large part, this is achieved thanks to epithelial cell division. As epithelial cells divide a new membrane interface forms between daughter cells. The formation of these new interfaces is controlled by tension forces generated by the intracellular network acto-myosin and transmitted by adherens junctions composed of E-Cadherin. Epithelia are mosaics tissues composed cells of distinct identities exhibiting different bio-mechanical properties and fulfilling the functions of a given epithelium. However, to date, the interplay between mechanical tensile forces and acquisition of cellular identity remains largely unknown.

We used dorsal thorax of *Drosophila* pupae, that consists in a single-layered neuroepithelium that produces only two types of cells, epidermal cells that divide symmetrically and sensory organ precursor cells (SOP) that divide asymmetrically to generate an anterior pIIb cell and a posterior pIIa daughter cell via segregation of the endocytic protein Numb, a cell fate determinant that behaves as a tumour suppressor gene inhibiting the Notch signalling pathway. Thus, the acquisition of cell identity is controlled by the differential activation of Notch. Notch is activated by the ligand Delta present on the surface of adjacent cells. It has been suggested that the regulation of receptor Notch and ligand Delta sorting along the apical-basal axis of epithelial cells during cytokinesis is a mechanism for controlling the activation of the pathway. In particular our previous studies suggest that Delta/Notch interaction is localized at the level of adherens junctions where mechanical tensile forces occur.

Dynamical analysis of new forming SOP daughter and epidermal cell interfaces show differences in the recruitment of myosin-II and Arp2/3 complex, suggesting mechanical tensile forces differences. To investigate interplay between Notch activation pathway and mechanical forces, we used laser nano-dissection to ablate newly formed adherens junctions and we measured the resulting relaxation of the network to evaluate the local ‘tension’ properties of junctions. We observed a progressive acquisition of tensile forces during cytokinesis, corresponding to a switch from a ‘low-tense’ to a ‘high-tense’ interface. Measurements performed on junction resulting of symmetric division present a ‘high-tense’ mechanical behaviour whereas pIIa/pIIb interface resulting of asymmetric division present a ‘low-tense’ mechanical behaviour. We are currently focusing on the mechanical role of regulators of membrane traffic, cell polarity, cell adhesion, acto-myosin contractility, to isolate the molecular mechanisms and associated mechanical properties related to Notch activation pathway. Our study aims to investigate the interplay between mechanical properties of interfaces and acquisition of cellular identity.

SNX27 interactome in T lymphocytes identifies ZO-2 dynamic redistribution at the immune synapse

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T lymphocyte recognition of antigens leads to the formation of a highly organized structure termed immune synapse (IS) by analogy with the nervous synapse. Sorting nexin 27 (SNX27) controls the endosomal trafficking of PDZ domain-interacting proteins, and its alteration is associated with impaired synaptic function and neurological diseases. In T lymphocytes SNX27 positive vesicles polarize to the IS, the identity of SNX27 interactors in these conditions nonetheless remains unknown.

Here we used proteomics to analyze the SNX27 interactome purified from IS forming T cells, and identified the retromer and the actin nucleator WASH complex, both essential for intracellular transport in neurons. Protein stability studies of SNX27 silenced T cells confirmed the conserved nature of the functional SNX27/WASH/retromer association in hematopoietic cells. Furthermore, our comparative analysis of the interactome of a SNX27 mutant with impaired PDZ cargo recognition identified the epithelial cell-cell junction protein zona occludens-2 (ZO-2) as an IS component. Biochemistry and microscopy approaches in T cells confirmed the presence of SNX27/ZO-2 interaction, and its role controlling the dynamic localization of ZO-2 at the IS.

This study broadens our knowledge of SNX27 function in T lymphocytes, and suggests that pathways that delimit polarized structures in nervous and epithelial systems also participate in IS regulation.

Intra- and intercellular trafficking of ephrins: the filopodia connection

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Alterations in different biosynthetic trafficking routes lead to losses in cellular polarity, which represent the earliest stages of carcinogenesis. We focused on the trafficking pathways of the tyrosine kinase receptor EphA2 and its membrane-bound ligand ephrinA1. EphA2 is frequently overexpressed in melanoma, gliomas, and around 10 different types of carcinomas where its levels are associated with poor prognosis. For instance in breast cancer EphA2 overexpression promotes tumorigenicity *in vivo* and its genetic ablation impairs tumor initiation and metastatic progression. In contrast, EphA2 activation by ephrinA1 acts like a tumor suppressor. Accordingly, manipulations that specifically decrease the EphA2 and not ephrinA1 levels at the plasma membrane are highly desirable for therapeutic interventions. The colocalization of EphA2 with the basolateral protein E-cadherin and the presence of an usually apical sorting motif (GPI anchor) in ephrinA1 strongly suggest that both proteins are segregated from each other in their journey to the plasma membrane. This may enables specific interventions of trafficking to uncover new pharmacological targets. To be able to study quantitatively and in real-time specific trafficking pathways our laboratory has developed a system called RUSH (Retention Using Selective Hooks), which allows to synchronize the secretion of virtually any protein.

By using the RUSH system, we demonstrated that EphA2 and ephrinA1 have different kinetics of transport and are partially sorted from each other at the level of the Golgi apparatus. Upon arrival to the plasma membrane, we observed that ephrinA1 clusters are preferentially generated at the tip of filopodia and seem to be found in the neighboring cells at later time points. We propose that ephrinA1 is transported from a sender cell that expresses the ligand, to a receiving receptor-bearing cell, throughout cytoneme-like signaling filopodia. Consistently, ephrinA1 clusters exclude other GPI-anchor proteins and are only formed upon contact of filopodia from the sender cell with a receiving cell. Subsequently, this may allow ephrinA1 transfer towards the receiving cell. Notably, we demonstrated that filopodia-localized clusters of ephrinA1 efficiently activate the Eph receptor on the receiving cells, which shows the functional significance of this behavior. Transfer by trans-endocytosis from the tip of signaling filopodia may thus occur in a two-step process that involves: i) cell-cell contact dependent clustering of ephrinA1 on the plasma membrane of the sender cell, and ii) actin-dependent trans-endocytosis of the full-length ephrinA1 from the signaling filopodia to the receiving cell. These data challenge the current model of shutdown of the ephrins type-A signaling which involve the ADAM-dependent cleavage and subsequent endocytosis by a receiving cell of the ephrin fragments and add a new level of complexity to the modulation of the long-distance signaling of ephrins in which filopodia length and dynamics could emerge as key regulators of the ephrin signaling.