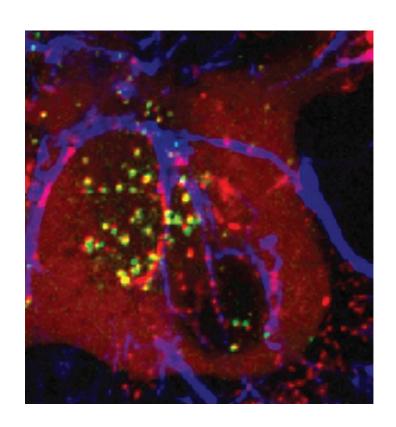




MOLECULAR BIOLOGY OF THE CELL

2014 - 2015



PROGRAM

MOLECULAR BIOLOGY OF THE CELL COURSE

2014 - 2015

(from 12th January to 13th February 2015)

Directors of the course

Roberto BRUZZONE

HKU - Pasteur Research Pole Hong Kong

Philippe CHAVRIER

Institut Curie - Research Section Paris

Head of Studies

Chiara ZURZOLO

Institut Pasteur Paris

Location

Centre d'Enseignement de l'Institut Pasteur Pavillon «Louis Martin» 28, rue du Docteur Roux 75724 Paris Cedex 15

Lectures: Room No 1 Practical Work: Room on the first floor

and

Amphithéâtre du Pôle de Biologie du Développement Institut Curie 11, rue Pierre et Marie Curie 75005 Paris

DESCRIPTION OF THE COURSE

The Molecular Biology of the Cell course is an intensive laboratory and lecture course of five weeks divided into weekly modules, each focusing on a cutting-edge aspect of cell biology. It is composed of lectures given by internationally renowned scientists, and of two practical sessions organized together with teams from the Curie and the Pasteur Institutes. The main topics of the course alternate between the cell biology of infection, cancer and intracellular trafficking emphasizing new experimental approaches. The availability of the core Imaging Platform at Institut Pasteur will introduce students to advanced techniques for the dynamic visualization of cells in health and disease.

The maximum number of participants is 24, which includes a selected number of Master 2 students from the University of Paris 6, Paris 7 and Paris XI and foreign postgraduate students in an interactive classroom setting. The course is intended to be a platform of excellence in which students can meet and closely interact with worldwide top-level scientists to discuss, exchange ideas and establish valuable contacts in the perspective of establishing a network of young cell biologists at an early stage in their careers. Students will be able to understand the importance of basic research and of a broad interdisciplinary approach to improve human health. We also expect to provide orientations and mentoring to help students in their future career.

The 2015 course is subdivided into four, week-long, modules. The first, lecture-only, provides a general introduction of cell organization and model organisms, with a focus on cell division, motility and tumorigenesis. The second and third weeks comprise both lectures and practical workshops addressing current topics and experimental approaches to investigate mechanisms of endocytosis and exocytosis, respectively. The fourth week will be devoted to the analysis of the experimental data obtained during the workshops, followed by dedicated sessions for oral presentation and discussion, chaired by an external expert in the field. At the end of this week, students not enrolled in postgraduate programs of Paris universities will have a final exam in the form of a journal club to critically analyze a recent paper related to the topic of the practical workshops. Master students of the Paris universities will have an exam at the end of the fifth week, consisting in the preparation of a written research project (4-5 pages max.) based on the follow-up of a recently published article. Students will then discuss their project in the final oral presentation. The overall evaluation is based on the participation in the course, the presentation of the practical work and the final exam.

Practical work 1

IMAGE ANALYSIS OF ENDOCYTOSIS

The endocytosis is an essential process based on the formation of vesicles from the plasma membrane that are then carried into other compartments such as early endosomes, recycling endosomes, lysosomes or Golgi complex. Immunofluorescence and microscopy are widely used to observe and analyze the different endocytic mechanisms. However, these technique needs to be associated to image analysis to be able to draw conclusion.

The goal of this workshop will be to initiate the students to image analysis software in the particular case of endocytosis. They will follow the intracellular fate of two cargos transferrin receptor (TfR) and interleukin 2 receptor (IL-2R) using immunofluorescent microscopy and Icy software to analyze their data to determine:

- Kinetics of TFR and IL-2R endocytosis upon time and temperature
- The sorting of each receptor towards several endosomal compartments.
- The robustness of the analyze at the statistical level

Practical work 2

ANALYSIS OF THE ANTEROGRADE TRANSPORT OF TNF USING THE RUSH ASSAY

In mammalian cells, about one-third of the newly synthesized proteins are destined to be secreted. Conventional secretory proteins enter the biosynthetic pathway at the level of the endoplasmic reticulum (ER), and they are then transported to the Golgi apparatus. From there, the secretory proteins are delivered to their final destination compartments (e.g., plasma membrane, extracellular medium, lysosomes) using post-Golgi transport carriers. During their transit through the Golgi complex, the proteins encounter posttranslational modifications such as glycosylation or proteolytic cleavage.

The RUSH assay will be used to synchronize the transport of a cargo, namely TNF (Tumor Necrosis Factor), of the secretory pathway. The aim of the different experiments is to evaluate the effects of chemical compounds on the transport of TNF. We will use 3 techniques: end-point assay using fixed cells and immunostaining, biochemical analysis using endoglycosidase H digestion and real-time imaging.

1st WEEK

THE ORGANIZATION OF THE CELL

 $Doctoral\ School\ module\ 1\ "Organization\ of\ the\ cell"$

Monday, 12th	January	Introduction
9:00 - 10:00	Welcome and administrative matters	Institut Pasteur Registration Office (Institut Pasteur, France)
10:30 - 12:30	<i>C. elegans</i> as a model organism to study morphogenetic forces	Michel LABOUESSE (IGBMC, France)
14:00 - 17:00	►Scope and overall course organization	Roberto BRUZZONE
	▶ Practical sessions with organizing teams	(HKU-Pasteur Research Pole) Philippe CHAVRIER
	▶ Presentation of the exam and paper assignm	(Institut Curie, France) nent Chiara ZURZOLO (Institut Pasteur, France)
17:00	Welcome party	
Tuesday, 13th	January	Cell division
9:00 - 11:00	Spatial and temporal control of cell division	Helder MAIATO (IBM, Portugal)
11:30 - 12:30	Control of asymmetric divisions in oocytes (part 1)	Marie-Hélène VERLHAC (College de France, France)
13:30 - 14:30	Control of asymmetric divisions in oocytes (part 2)	Marie-Hélène VERLHAC (College de France, France)
15:00 - 17:00	Cell migration under confinement: pushing off the walls and squeezing the nucle	Matthieu PIEL eus (Institut Curie, France)
17:00 - 18:00	Students' self-presentation	
Wednesday, 1	4th January	Cellular and subcellular motility
9:00 -11:00	From cell to tissue: mechanisms of epithelial morphogenesis	Yohanns BELLAICHE (Institut Curie, France)
11:30 - 12:30	Mechanisms of nuclear positioning within cells (part 1)	Edgar GOMES (Instituto de Medicina Molecular, Portugal)
13:30 - 14:30	Mechanisms of nuclear positioning within cells (part 2)	Edgar GOMES (Instituto de Medicina Molecular, Portugal)
15:00 - 17:00	Leukocyte adhesion and migration through endothelial cells: old players, new puzzles	Ronen ALON (Weizmann Institute, Israel)
17:00 - 18:00	Students' self-presentation	

Thursday, 15th January		Model organisms
9:00 - 11:00	Synapses, secretion and cilia	Gillian GRIFFITHS (Cambridge Inst. Medical Research, UK)
11:30 - 12:30	The collective cell biology of organ formation (part 1)	Darren GILMOUR (EMBL, Germany)
13:30 - 14:30	The collective cell biology of organ formation (part 2)	Darren GILMOUR (EMBL, Germany)
15:00 - 17:00	Collective migration of neural crest cells	Roberto MAYOR (University College London, UK)
17:00 - 18:00	Students' self-presentation	

Friday, 16th January Cancer

	INSTITUT CURIE DAY Location : Amphithéâtre du Pôle de Biologie du Développement Institut Curie 11 rue Pierre et Marie Curie 75005 Paris
10:00 - 12:00	Endocytosis and signaling Pier Paolo DI FIORE (FIRC Inst. of Molecular Oncology Foundation, Italy)
12:30 - 14:00	Lunch for students and speakers - Hall du Pôle de Biologie du Développement
14:00 - 15:00	Cancer invasion and the microenvironment Danijela MATIC VIGNJEVIC (Institut Curie, France)
15:00 - 16:00	Mechanism of Matrix Metalloproteinase secretion during breast tumor cell invasion Philippe CHAVRIER (Institut Curie, France)

IMAGING ENDOCYTOSIS

Doctoral School module 2 "Membrane mechanics of endocytosis"

PRACTICAL WORK 1

IMAGE ANALYSIS OF ENDOCYTOSIS

under the direction of Nathalie SAUVONNET, Thibault LAGACHE, Fabrice DE CHAUMONT and Guylène K'OUAS

Monday, 19th January		
9:00 - 11:00	Coated vesicles and intracellular trafficking	Margaret S. ROBINSON (CIMR, Univ. Cambridge, UK)
12:00 - 13:00	Seminar BCI Coated vesicle adaptors	Margaret S. ROBINSON (CIMR, Univ. Cambridge, UK)
14:00 - 15:45	Introduction to practical 1 "Image analysis of endocytosis"	
16:00 - 18:15	Practical work 1 Day 1: Cell plating on coverslips	
Tuesday, 20th	ı January	
9:00 - 10:30	The clathrin-independent endocytosis	Nathalie SAUVONNET (Institut Pasteur, France)
10:30 - 12:00	Bioimage Informatics for High Content Screen	Thomas WALTER (Institut Curie, France)
13:30 - 18:30	Practical work 1 Day 2: Endocytosis by immunofluorescence	
Wednesday, 2	1st January	
9:00 - 10:30	Quantitative Bioimage Analysis	Jean-Christophe OLIVO-MARIN (Institut Pasteur, France)
11:00 - 12:15	Image archival, intelligent retrieval and knowledge discovery in image databases	Perrine PAUL-GILLOTEAUX (Institut Curie, France)
13:30 - 18:30	Practical work 1 Day 3: 13:30 - 16:00 Epifluorescence - Binomes: 1-5 ICY initiation - Binomes: 6-10 16:00 - 18:30 Epifluorescence - Binomes: 6-10 ICY initiation - Binomes: 1-5	

Thursday, 22nd January

9:00 - 12:00	Membrane Traffic and cytoskeleton remodelling during cell division	Arnaud ECHARD (Institut Pasteur)
13:30 -18:30	Practical work 1 Day 4: Image analysis	

Friday, 23rd January

9:00 - 11:00	Mechanisms for the acquisition of cell polarity during epithelial morphogenesis	Fernando MARTIN-BELMONTE (CBM CSIC, Spain)
12:00 - 13:00	Seminar BCI Endocytosis regulates tissue patterning in epithelial tubular organs	Fernando MARTIN-BELMONTE (CBM CSIC, Spain)
14:00 - 18:15	Practical work 1 Day 5: Image analysis	

3rd WEEK

DYNAMICS OF EXOCYTOSIS

Doctoral School module 3 "Endo-exocytosis and Image Analysis"

PRACTICAL WORK 2

ANALYSIS OF THE ANTEROGRADE TRANSPORT OF TNF USING THE RUSH ASSAY

under the direction of Franck PEREZ, Gaëlle BONCOMPAIN, Séverine DIVOUX and Guylène K'OUAS

Monday,	26th	January
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9:00 - 11:00	Single-molecule biology and visualization of endocytosis	Tomas KIRCHHAUSEN (Harvard Medical School, USA)
12:00 - 13:00	Seminar BCI Dynamics of endocytosis	Tomas KIRCHHAUSEN (Harvard Medical School, USA)
14:00 - 19:00	Practical work 2 Day 1: Cell seeding for experiments 1 & 2, acrylamide 1st series of trafficking movies	gels preparation for experiment 2

Tuesday, 27th January

900 - 12:00	Dynamics and functions of the Golgi complex	Fr
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Franck PEREZ (Institut Curie, France)

13:00 - 19:30 Practical work 2

Day 2: Induction of transport of cargos and immunofluorescence (experiment 1), transfection (experiment 2)
2nd series of trafficking movies

Wednesday, 28th January

9:00 - 12:00	Image formation and digital microscopy	Phong TRAN
		(Institut Curie, France)

13:00 - 19:30 *Practical work 2:*

Day 3: Observation (experiment 1), induction of trafficking and lysis (experiment 2), 3rd series of trafficking movies

Thursday, 29th January

9:00 - 11:00	The role of post translational modifications	Sumana SANYAL
	in immunity and infection	(Hong Kong University, HK)
13:00 - 19:00	Practical work 2 Day 4: Biochemical analysis of transport and western blot 4th series of trafficking movies	t (experiment 2),

Friday, 30th January		
9:00 - 10:30	Single event analysis of clathrin mediated endocytosis	Christien MERRIFIELD (LEBS CNRS, France)
1030: - 12:00	Lysosomal signaling and disease	Matias SIMONS (Institut Imagine, France)
14:00 - 19:00	Practical work 2 Day 5: End of immunoblot (experiment 2), 5th series of trafficking movies	

4th WEEK

GROUP ANALYSIS OF EXPERIMENTAL DATA AND PRESENTATION OF THE PRACTICAL WORKSHOPS

Monday, 2nd February

9:00 - 11:00 ECM, Skin and mineralised bones: Vivek MALHOTRA

Mechanism of collagen secretion (CRG, Spain)

12:00 - 13:00 Seminar BCI Vivek MALHOTRA

Secretion of proteins that cannot enter the (CRG, Spain)

ER-Golgi pathway

14:00 - 19:00 Analysis of Practical Work 2

Tuesday, 3rd February

9:00 - 12:00 Analysis of Practical Work 2

13:30 - 17:30 Presentation of Practical Work 2

Wednesday, 4th February

9:00 - 18:15 Analysis of Practical Work 1

Thursday, 5th February

9:00 - 12:00 Axonal transport in health and disease Giampietro SCHIAVO

(UCL, UK)

13:30 - 17:30 Presentation of results per group of Practical Work 1

Friday, 6th February

10:00 - 12:00 Oral exam for non Master students

14:00-16:00 Oral exam for non Master students

5th WEEK

PROJECT SUBMISSION AND EXAMS

Monday 9th to Tuesday 10th February

Project preparation. The written project has to be submitted to the Course Committee by Tuesday night.

Wednesday 11th to Thursday 12th February

Preparation of the oral exam

Friday, 13th February

9:00 - 18:00 Final examination

Detailed description on next page

18:00 Closing party

DETAILED DESCRIPTION OF THE EXAMINATION

Oral exam on Friday 13th February, 2015 (mark on a 1-20 scale, coefficient 1):

<u>Critical analysis of a scientific article and presentation of an imaginary 3-year research project as follow-up of the results of the article.</u>

Presentation: 13 minutes, questions: 7 minutes, total duration: 20 minutes

Organization of the oral presentation:

- The presentation is open to the public
- Slides (Powerpoint or other supported formats)

The scientific articles will be given to students during the first week of the course. Each student will write a fictional project intended to be a follow-up of the article received and submit a 4/5 page document to the members of the jury no later than Tuesday 12 February at 20:00. This document should include:

- Summary of the article (max 1 page)
- Aims and description of the project (max 3 pages including figures if appropriate)
- References (max 1 page), using the style of a cell biology journal (e.g., JCB, JCS, MBC, Cell, NCB....)

DESCRIPTION DETAILEE DE L'EXAMEN

Examen oral le vendredi 13 février 2015 (note sur 20, coefficient 1):

Présentation critique d'un article et discussion d'un projet fictif sur 3 ans découlant de ces résultats.

Présentation: 13 minutes, questions du jury: 7 minutes, durée totale: 20 minutes.

Organisation de la présentation orale :

- Exposé public de chaque étudiant devant le jury
- Diapositives (logiciel Powerpoint ou autre format compatible)

Les articles scientifiques seront donnés aux étudiants pendant la première semaine de cours. Le projet fictif est présenté dans un document de 4/5 pages à remettre au jury au plus tard le mardi 12 février à 20h00, comprenant :

- Résumé de l'article (max 1 page)
- Objectifs et description du projet (max 3 pages, figures incluses)
- Bibliographie (max 1 page) selon le style d'un journal type JCB, JCS, MBC, Cell, NCB...





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2014 - 2015

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