

Intracellular signaling in normal and pathological conditions

Coordinator: Jacques Ghysdael
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Objectives. The deregulation of intracellular signaling pathways that govern self-renewal of stem cells and the proliferation/survival/differentiation control of progenitor cells is at the heart of oncogenic processes. The first objective of this Unit is the description of (i) the role of critical pro-oncogenic signaling pathways during normal development ; (ii) the molecular players involved in these different pathways and their relationships; (iii) how the deregulation of these pathways contributes to oncogenic processes. The second objective of this Unit is to describe how - at the experimental level - the dissection and mode of action of signaling pathways can benefit from the use of complementary biological systems, in particular animal models. This Unit will be associated with several practical workshops in the laboratories and technological platforms of the Institut Curie, in which the techniques currently used to analyse the activity of signaling pathways at the cellular and molecular levels and their deregulation in cancer will be studied.

Duration and location : 1 week at the Institut Curie, Centre Universitaire, Bat110, 91405 Orsay, France. The Unit is open to 30 students this year.

For additional info, www.curie.fr/recherche/themes/detail_unites.cfm/lang/fr/id/46.htm

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PROGRAM.

1. Signaling pathways (theoretical lessons; 12hours 30 min)

Welcome address/Introduction (*Jacques Ghysdael, I. Curie*)

The Wnt/beta-catenin pathway (*Lionel Larue, I. Curie*)

The Sonic Hedgehog pathway (*Nadia Dahmane, Wistar Institute, USA*)

The Notch pathway (*Neetu Gupta, I. Pasteur*)

The TGFbeta/BMP pathway (*Celio Poupponnot, I. Curie*)

The JAK/STAT pathway (*Jacques Ghysdael, I. Curie*)

The PI3K/AKT pathway (*Frédéric Saudou, I. Curie*)

The RAS/RAF/MAPkinase pathway (*Alain Eychène, I. Curie*)

The receptor-type protein tyrosine kinases (*Serge Roche, CNRS, Montpellier*)

FGFR signaling in development and cancer (*François Radvanyi, I. Curie*)

2. Experimental approaches I (theoretical/technological lessons; 8 hours)

Protein-protein interactions and post-translational modifications: a proteomic approach (*Philippe Marin, CNRS, Montpellier*)

The cell: its approach through imaging (*Fabrice Cordelières, I. Curie*) ; this lesson will be associated with a visit to the Cellular Imaging platform of the Institut Curie.

3. Experimental Approaches II (Laboratory Workshops; 15 hours)

This activity consists in practical workshops in the Laboratory of Cellular Interactions and Oncogenesis, UMR146 CNRS/Institut Curie and the technological platforms of the Institut Curie. During these workshops, participants (4 max. per workshop) will become accustomed and will use the technologies and biological systems (cellular and animal systems) used to analyse specific signaling pathways. The choice of the different workshop will be done on the first day of the program.

A few examples of the practical workshops :

Workshop #1 : Cellular and Molecular Analysis of Signaling Pathways

(C. Tran Quang ; J. Ghysdael)

This workshop proposes to follow the cellular response (self-renewal; survival/apoptosis ; differentiation) of erythroid progenitors to a cytokine (Epo) and the activation of the JAK/STAT pathway and to stem cell factor (SCF) through its binding to the c-Kit receptor tyrosine kinase. The perturbations imposed by specific oncogenes upon these pathways will also be analyzed. This study will be complemented by biochemical analyses to demonstrate the activation of specific molecular players involved in cytokine and growth factor receptor signaling.

Workshop #3 : In vivo and in vitro Analysis of the Wnt/Beta-catenin Signaling Pathway in *X. Laevis* (A.H. Monsoro)

This workshop will illustrate the analysis of a signaling pathway through *in vivo* microinjections and organ culture in *Xenopus* embryo. The Wnt/beta-catenin pathway will be activated or inhibited through the overexpression of specific genes and through the use of morpholino antisense RNA. This workshop will illustrate the efficiency of *Xenopus* as a model system to demonstrate the involvement of specific components of a signaling pathway critical during normal development and found to be deregulated in specific cancers.

Workshop #6 : From the Production to the Histological Analysis of Transgenic Mice
(L. Larue)

The aim of this workshop is to show students the key steps of the techniques used to generate a transgenic mouse and to study transgenic mice at the histological level. This workshop will be divided in three phases: 1) the production of recombinant embryonic stem cells (ES cells) ; 2) the production of transgenic animals through pronuclear injection of DNA and through injection of ES cells in the blastocyst ; 3) the principles of histological analysis.

Workshop #7 : Analysis of Intracellular Dynamics as analyzed by 3D Microscopy and Videomicroscopy
(F. Cordelières)

The aim of this workshop is to acquire the basis required to study the movements of cell membranes, organelles or specific proteins in cell lines or primary cell cultures. The following techniques will be demonstrated and used during this unit: protein labeling in fixed and living cells, cell microinjection, 3D microscopy and deconvolution image analysis, 3D videomicroscopy and movements analysis.

DIRECTIONS TO INSTITUT CURIE, ORSAY

To reach Orsay, **use the RER system**. This is a train linked to the metro system which connects Paris to the suburbs. A map of the entire system can be obtained in RER/metro stations.

Use the RER-B line (stations in Paris are at **Gare du Nord, Les Halles, Notre Dame, Port Royal, Luxembourg and Denfert-Rochereau**). You have to take a train heading south **with end station Saint-Rémy -les -Chevreuse**.

Be careful, the B line heading south is **Y-shaped**. Some trains have **Saint-Rémy-les -Chevreuse** as terminus, others have Robinson as end station. **Only use those having Saint-Rémy-les -Chevreuse (or Orsay-Ville) as terminus**.

DO NOT use those heading to Robinson.

Direction informations are clearly indicated on electronic timetables hanging from the ceiling of the platforms. Once in the right train, it will take about 35 minutes to reach Orsay from Denfert-Rochereau. **All trains** with Saint-Rémy -les -Chevreuse as terminus will stop in Orsay.

Step down at Orsay-Ville. The Institute is at a 5 min. walking distance from the station

The Institute Curie is on the campus of " Université Paris Sud ". Topologically, it is localized facing the RER station of Orsay-ville (but on the other side of the railway tracks that you cannot cross).

At the exit of the RER station, walk down the tunnel which is running under the railway tracks. Turn left in the street and walk down. Pass the first intersection on your left, and continue to walk down until reaching a large two ways street with trees in the middle and a river on the right. Turn left in that street (this is 100 meters from the station). Never cross the river (means you are too far). After about 50 m, you will reach the entrance gate (security office, open during the day). Pass it an keep walking straight (do not turn left, because the road is closed due to roadworks). Keep walking straight for about 200 meters. At the building carrying the inscription « Soleil » on its wall, stay on its left and keep walking on the main road. It will zig-zag a couple of times and will bring you after 100 m in front of **building 111** of the Institut Curie. The amphitheater is in building **111** (at the 2nd floor). Our lab is in **building 110 floor level**.

In case of problems, please phone our secretary **01 69 86 71 93/**,
or my lab **01 69 86 30 27**, or my office **01 69 86 31 52**, or the general number **01 69 86 30 00**

Address of the lab :

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91405 Orsay