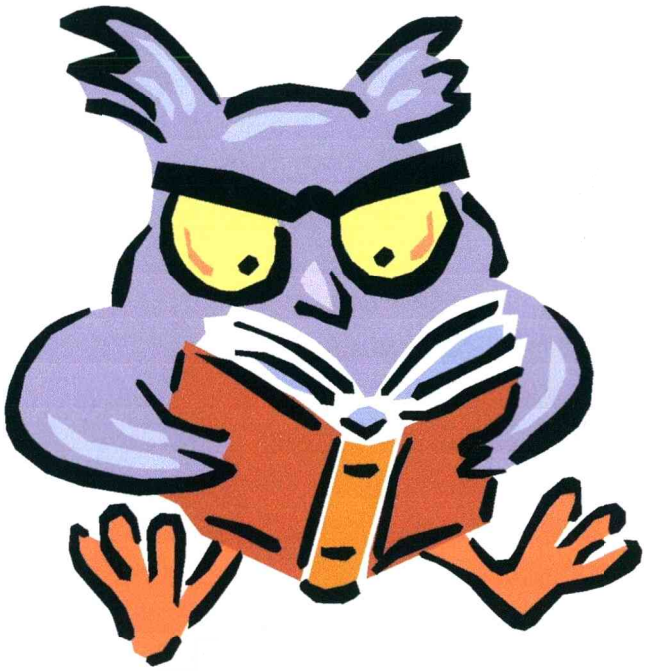
Topical Day – December 11, 2001

Topical Day on Molecular Biology at the post-genomic era as a tool in nuclear research



SCK•CEN
Belgian Nuclear Research Centre
Boeretang 200
B-2400 MOL

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Preface

Every compartment of the biosphere from deep-sea hydrothermal sources to atmospherical aerosols, from bare soils to luxuriant equatorial forests, from hot springs to Antarctic dry valleys, from crop fields to hospitals or to industrial environments, from bacteria to man, is now addressed by the enormous databases generated by the genomic effort. The tools to process these huge amounts of information are still in their prime youth and a lot of questions remain unanswered about cancer, infectious diseases, hereditary diseases, or genetic damages induced by ionising radiations, to quote only a few questions from medicine and public health area.

The objective of this topical day offers the opportunity to learn about the latest developments in molecular biology in the post-genomic era and their potential involvement in a Nuclear Center as **SCK•CEN**, *mainly in the field of radiobiological sciences and radioprotection (diagnostic and radio-therapeutic applications) but also in the field of environmental sciences (long-term ecology of the storage of radioactive wastes, bioremediation...).*

Bacterial genomics

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Abstract

Microbes have evolved for several billion years in such a way that they have colonized almost every ecological niches, including the most adverse such as an extreme temperature, a high level of radiations or the presence of toxic compounds. In addition, their complex regulatory systems allow microbial cells to adapt to rapid changes in their environmental conditions. Understanding the organization and the regulatory processes of microbial cells will allow the exploitation of their beneficial features. Indeed, microorganisms have developed an extremely diverse collection of capabilities that can be used in toxic-waste cleanup, energy production and biotechnology. During the past 15 years, the development of genomic DNA sequencing allowed the publication in 1995 of the first complete genomes of bacterial microorganisms and of baker's yeast *Saccharomyces cerevisiae*. These works revealed our lack of understanding of living organisms, including model organisms such as the enteric bacterium *Escherichia coli* and the soil bacterium *Bacillus subtilis*, which have been extensively studied. Indeed, the function of more than 30% of the predicted genes remains unknown. To date, the complete sequence of about 60 microbial genomes is available and about 200 more are currently being sequenced. At the same time, these programmes of genomics have lead to an explosion of technological advances in various domains such as automation and bioinformatics and gave rise to new methods, the so-called genome-wide or large-scale technologies. Two-dimensional electrophoresis – which allows protein separation according to their size and charge in total protein extracts – coupled to mass spectrometry – which makes possible the characterization of polypeptides separated in a gel – can be used to identify proteins present in an organism under specific growth conditions. Moreover, DNA arrays or chips – which are nylon membranes or glass slides (up to 1 cm^2 in size) where all the genes of an organism can be spotted – allows to evaluate their expression level and to study the mechanisms required for bacterial adaptation in response to various stresses. Proteome and transcriptome are powerful methods to address the physiology of an organism in a global way and constitute therefore the basis of global approaches toward the understanding of regulatory cellular networks. Our concepts for studying biology are now shifting from the study of individual protein or gene to that of global expression patterns. These approaches represent an important step in moving from cataloguing molecular parts to constructing an integrative view of live. Gaining an understanding of the complexity of living systems requires new ways of thinking and collaborations with scientists from disciplines such as engineering, chemistry and physics. This should be of major importance to understand how life's components function together and are influenced by environmental factors in creating dynamic living systems. In particular, such approaches should be extremely useful to identify in *Deinococcus radiodurans* (a radioresistant bacterium whose genome has been sequenced 2 years ago) or in *Ralstonia metallidurans* (a metalloresistant microorganism whose genome sequencing is in progress) new functions and their regulatory mechanisms, which could play an important role in bioremediation of polluted environments.

Proteomics: a way to functional genomics

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Abstract

Genome sequencing being nowadays routinely executed, with genome sequences of over 800 organisms completely or progressively available on NCBI website in November 2001, the interest of the scientific community has moved towards the extraction of any information embedded in these huge amounts of DNA sequences, entering the postgenomic era. Among the wide variety of tools available for functional genomics, proteomics holds a key position since proteins are the functional output of the cell and may therefore provide the most relevant information, especially since interpretation of their expression levels integrates many steps including transcriptional control, alternative splicing, translational control, posttranslational modifications and degradation. The proteomic technology comprises a plethora of techniques to isolate, resolve, quantify and identify proteins of microbial origin as well as from eukaryotes. However, without applications, proteomics would just be technology. Illustrations of cell-map proteomics, focused on the depiction of protein networks within the cell and expression proteomics, devoted to the quantitative analysis of protein expression from cells prepared in different physiological states (disease, environmental stress ...) will be provided.

Meeting the challenges of the Post Genomic era: the role of bioinformatics

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Abstract

A hundred of so complete genomes, of species ranging from bacteria to man, have now been sequenced, and many more are in the pipeline (Web site NCBI Genomes). This flow of information is changing the way in which research in all fields of biology is performed. Until recently most biochemists and molecular biologists focused on the properties of single genes and proteins, involved in individual biological processes. Now, it becomes possible to study how the individual genes and gene products co-operate to build up complex cellular structures and to perform all the elaborate processes that enable cells and organisms to live and reproduce themselves. But before this vast new potential can be exploited, the genome sequence information must be decoded in terms of biological function. This endeavour, termed Functional Genomics requires a concerted effort involving theoretical and experimental approaches. The experimental approaches aim at characterising the patterns of gene and protein expression at the levels of entire cell (*transcriptome and proteome* analyses respectively), as well as the full complement of the protein-protein interactions, believed to be responsible for the complexity of living organisms. They also endeavour at determining the three-dimensional structures of all the proteins in a cell or organism (*structural genomics* or *structural proteomics*), or at systematically characterising the biological processes in a cell .

The theoretical approaches, broadly denoted as 'bioinformatics', cover a very large spectrum of methods and even disciplines. Most classically, they represent techniques and methods for managing the momentous amount of data on gene and protein sequences, and the much more complex data and information on the corresponding biological function. In recent years these approaches are being expanded to the interpretation of gene expression data, or of the cellular protein complement, as well as to the prediction of structure and function from sequences and the prediction of function from protein 3D structures.

This presentation will aim at providing an overview of what the bioinformatics approaches encompass, and will illustrate their application to the analysis of microbial genomes.

Biotechnology in a nuclear environment

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Abstract

Nuclear programs may rely on molecular biology applied to microorganisms: the following topics are now addressed by the current programs of SCK/CEN in Mol:

-the microbiology of the geological sites designed to become long-term repositories of radioactive waste : it is of importance to describe the established microbial communities and to check if they could affect the materials in which the radioactive waste was conditioned : sulfate-reducing bacteria, metal-resistant heterotrophs, acidophiles, methanogens, bacteria, thermophiles are among the groups of interest in this respect...

-the microbial ecology of soils or sediments submitted to long term exposure to radionuclides : fall-out, radioactive wastes, uranium mining areas, sludges from nuclear power plants...These nuclear biotopes can also be the sources of new radiation-resistant microorganisms, which are specifically adapted to survive in such harsh environments and could be used for bioremediation.
-the bioremediation of radioactive wastes (solid or liquid), soils or sediments using a combination of microorganisms specifically adapted to radiations , resistant to heavy metals or able to degrade recalcitrant organics (especially in the perspective of mixed wastes that are a major source of environmental concern)
-the effect of cosmic radiations on the genetic stability of microorganisms growing in bioreactors used in space vehicles for waste recycling or food production.
To address these various topics, molecular biology is a major tool : a major difficulty resides in the isolation , the cultivation and the identification of the various microbes of the microbial communities : cultivation methods on Petri dishes are giving only a scarce view of the existing microbial diversity . Total extraction of DNA followed by appropriate amplification and the full sequencing of key DNA sequences (via the PCR technologies (Polymerase Chain Reaction)) allowed to make a much better description of the studied communities in the relevant biotopes and to identify key microbial actors.

On the other hand, the genomics and the proteomics of the bacteria adapted to extreme physico-chemical conditions will help to make a catalogue of genes and functions involved in the response and the tolerance to ionizing radiations, in the degradation of recalcitrant compounds and to the detoxification of heavy metals.

In this respect, much attention has been given in Mol to the genomics, the proteomics and the transcriptomics (via quantitative RT-PCR) of *Ralstonia metallidurans* for which the draft sequence of the genome is now available. This bacterium was isolated from industrial biotopes highly polluted by heavy metals and. It contains two large plasmids (pMOL28 : 170 kb and pMOL30 : 250kb) that contains a multiplicity of genes and operons involved in the resistance to at least 10 heavy metals. The study of this bacterium has led to some environmental applications as biosensors, bioreactors designed to remove heavy metals from polluted soils or effluents, treatments of mixed pollutions and phytoremediation.

All you wanted to know about MicroArrays

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Abstract

With the human genome project reaching its completion scientists are looking for new ways to process the sequence data to functional knowledge of the genes in biological processes. During the last 5 years a new technique, MicroArrays/DNA-Chip, immersed that allows to studying gene expression on a very large scale (>10,000 genes at once).
Recently, VIB initiated a central core facility for microarray analysis with the state of the art technology. Currently, the facility is routinely analyzing samples from different lines of research and has the capacity to analyze 16,000 human, 22,000 mouse and 4,500 Arabidopsis genes.
Along with microarrays a new problem and consequently a new field immersed which is bioinformatics. Biologists are suddenly faced with data files that easily contain 10-20 columns of measurements each 200,000 rows long that express results of 5,000 to 60,000 potential genes in several different states. Analyzing such large data files are major hurdles for common biologists to interpret the data and, therefore, there is pressing need for help and input of informatics, mathematics and statistics.
In this lecture we will cover the basics of microarray production and analysis and the existing tools to analyze large datasets.

Alteration of the profiles of gene expression in virally-and radiation-induced apoptosis

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Abstract

Ionising radiation (IR) can in one hand, induce some types of cancers. The underlying mechanism might be through the activation of an oncogene or inactivation of a tumour suppressor gene. The altered activity of these genes might result from chromosome rearrangements, point mutations, or changes in their expression levels. In the other hand, IR can trigger cell death either by apoptosis or reproductive death. The molecular responses to IR are complex and might involve a variety of regulatory pathways. One key element in the cellular response is the transcription factor p53, which regulates several genes involved in cell cycle progression and apoptosis. Its role in cancer is evidenced by the fact that it is inactivated in most human tumours, and that it is also neutralised by oncogenes from cancer-inducing viruses, such as the papillomavirus type 18 (HPV18) E6 protein. Besides its involvement in carcinogenesis, p53 plays also a role in development, as p53 deficiency results in developmental abnormalities (predominantly defects of neural tube closure).

In order to get insight into the pathways activated by IR and to elucidate the role of p53 in these molecular changes, we have focused our research activities on the study of global gene expression differentially induced in three experimental systems, namely 1) apoptosis of human cells induced by the viral HPV18 E2 protein, 2) response of different lineage of human hematopoietic cells (both cancerous and normal) after IR, 3) response of the developing mouse brain after IR and role of p53 in this process.

Global gene expression is being studied essentially with DNA micro-array technology, and to other extent, with subtractive-suppressive DNA hybridisation (SSH). The type of radiation being studied corresponds to 250 KeV x-rays, and the doses we are delivering (0.5 to 8 Gy) correspond to those that normal tissue or solid tumours could receive in fractionated radiotherapeutic treatment, or in case of a nuclear accident or a clinical whole body irradiation.

The mechanism of HPV18 E2-induced apoptosis has been studied by infecting HeLa cells (constitutively expressing the viral HPV18 E6 and E7 oncogenes) with a recombinant adenovirus expressing various mutants of the E2 protein linked to the green fluorescent protein (gfp). Just before cells underwent apoptosis, cells were harvested, and their corresponding cDNA labelled and hybridised to 3 different microarrays containing each +/- 4606 ESTs spotted in duplicate (VIB microarray facility). Out of 13818 genes on the microarrays, 2.5% were repressed and 1% were activated more than 2.5 fold by E2. More than 40 of these genes were picked up for validation with real-time quantitative

RT-PCR. In general, there was a good agreement between the two techniques. The HPV18 E6 was repressed more than 20 fold, while the p53-inducible p21 cell cycle inhibitor was activated more than 30 fold, confirming previous observations. Clustering of genes in functional categories allowed to draw some general conclusions as to the role of E2 in the viral life cycle or in apoptosis. First, apart from PIG3, the expression of the other PIGs (involved in p53-induced apoptosis) remained unchanged, suggesting that E2-mediated apoptosis is at least partly independent of p53, although some of the p53-inducible apoptotic genes were moderately overexpressed, such as Bak1 (3 fold) or Bax (6 fold). Alternative pathways might include PTFG-beta which is activated 10 fold by E2. Second, E2 down-regulates genes from different pathways involved in the immune response, creating therefore a way to escape to the anti-viral response of the host cells. Third, E2 seems to change the expression of various genes involved in the structure of the epithelial cells, thereby possibly interfering with the normal differentiation of the host cell and making the release of the virion easier.

At present, the effect of IR on hematopoietic cells has been tested on the JURKAT cell line and one normal T clone with a total of 5 human VIB microarrays. The role of p53 in IR-induced developmental abnormalities in the brain will be assayed on p53-/-and p53wt mice irradiated during developement.

We will thus present data validating the technique of microarrays for the identification of pathways altered by p53 and ionising radiation.

Functional bioinformatics of microarray data: from expression to regulation

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Abstract

Unraveling the mechanisms of gene regulation is currently one of the major challenges of bioinformatics. Towards this goal, we integrate both clustering of microarray data and detection of candidate regulatory elements from clusters of coexpressed genes.

Firstly, we address a number of shortcomings of classical clustering algorithms with a new method called adaptive quality-based clustering in which we look for tight reliable clusters.

Secondly, we introduce an extension of the Gibbs Sampling algorithm for motif finding that uses a higher-order background model to improve the detection of motifs in noisy data sets. These algorithms are available through our INCLUSive web tool (http://www.esat.kuleuven.ac.be/~dna/Biol/Software.html), which performs all the data analysis steps semi-automatically and provides extensive support to the user.

Contemporary microbial genomology

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Abstract

More than five years after completion of the sequence of the yeast genome, I will define the object of contemporary genomics and of the different activities exerted under this name. In particular, I will illustrate the contribution of phylogenetics in the biochemical exploration of the numerous new genes encoding membrane proteins. I will give as example the analysis of the large families of P-type ATPases and multidrug -ABC transporters in yeast species

I will raise the prospect of structural and biochemical analysis unlashed by the *in silico* discovery of more than 25,000 new membrane proteins revealed these last five years by the systematic sequencing of more than 100 species, mostly microbial. Today, the bottle neck is still the heterologous overexpression of membrane proteins from species which have limited *in situ* potential for genetic and/or physiological manipulations.

I will describe achievements and problems met by the use of yeast for heterologous expression of animal, parasite, plant and archae membrane proteins. In this view, the potential of yeast for expression of extremophiles will be raised.

Modern microbial ecology benefits from the advances made in molecular biology, and it now has become possible to analyse the composition and dynamics of microbial populations in air, soil and aquatic environments, with a special attention to gene rearrangements and dissemination. In this respect, an important topic is to observe the relationships between sturdy inhabitants of soils and the opportunistic pathogens that are able to overcome barriers in hospital and clinical environments.

A new area in microbial ecology is now opening up with the study of 'extremophiles': microbes that are able to survive extreme pH, temperature, pressure, or brutal chemical assaults. The study of extreme and harsh environments (whether natural or anthropogenic) paves the way to the exploitation of new microbial resources in terms of such diverse areas as bioremediation, "biohydrometallurgy", and space research. Extremophiles also offer perspectives to model systems for exobiology (e.g. life on Mars) or the development of life support systems for long term space travel.

One major and for a long time completely overlooked lesson of the industrial revolution is that the microbial world has coped with huge environmental aggression derived from human activities in a relatively short period of time. In doing so, it has revealed a tremendous capacity to evolve, thereby challenging some aspects of the current theories of evolution. We need to know more about these capabilities in order to rationally exploit the vast microbial resources as well as to counteract the undesired effects that may have arisen as a consequence of human mismanagement.

Mutual Interactions between the Living World and Urbanised Societies shaped by the Industrial Revolution.

Some Microbial Points of View

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The industrial revolution from the last three centuries has given an enormous momentum to the urbanisation of our planet. The synergy between urbanisation and industrial revolution has put worldwide an enormous burden on the vegetation and animal life.. Though the Life sciences have influenced this synergy as observed from the development of intensive agriculture and husbandry, the biotechnological breakthroughs, and an improved health care, the bottom line of it all is that the overall landscape of our planet has been dramatically, and perhaps irreversibly, changed.

Yet a correct appreciation of the underlying complex interactions between urbanised societies and the living world should also take into account the hidden part of the biosphere: the microbial world, a world so vastly important in shaping the environmental conditions in which plants can grow, animals breed, and human societies survive.

Among the countless interactions between the microbial world and present time human activities or environments, special interest goes out to soil microbes and their reaction to the huge release of chemicals and industrial wastes. One such organism is *Ralstonia*, in particular *Ralstonia metallidurans*, a bacterium that is specialised in colonising metallurgical biotopes that are severely contaminated by heavy metals, often together with man-made organo-chemicals.

The dissemination of antibiotic resistance determinants attracted the attention towards transmittable or mobile genetic elements such as plasmids, phages, and transposons. In due course, related groups of these genetic elements were found to actually carry genes providing responses to 'brutal' environmental changes, such as the presence of heavy metals. Because transmission of these genes also occurs across different genera of bacteria, such genes are often referred to as belonging to the "horizontal gene pool". Remarkably, these resistance genes as well as the whole arsenal of the "horizontal gene pool" has provided powerful tools for biotechnological research. In fact, current biotechnology is mainly based on microbes even in the application to plant biotechnology or to the production of various therapeutical products.

Remarkable adaptations in microbes were also triggered by the huge release of recalcitrant man-made chemicals such as herbicides or pesticides and not for long, soil microbes learned to use these 'xenobiotic' compounds as sources of carbon or nitrogen.

Again, it became possible to track down the genes confering such new abilities. In addition, modular combination of genes within the mobile genetic elements led to the emergence of new genes or at least to the extension of existing gene capabilities in response to new environmental conditions or substrates.