IIIeme cycle romand de sciences biologiques

Symposium

Homologous recombination and functional genomics in plants

Les Diablerets, June 29th –30th 2000

Organisers: Didier Schaefer and Jean-Pierre Zrÿd

Institute of Ecology, Plant Cell Genetics,
University of Lausanne, Switzerland

PROGRAM

Thursday June 29th

13.00

Arrival and registration

14.00

Dr. Ivan MATIC

"Homeologous recombination, horizontal gene transfer and bacterial evolution"

Faculté de Médecine Necker, Université de Paris V, France

15.00

Dr. Neal SUGAWARA

"Genetic requirements for break-induced recombination in *S.cerevisiae*"

Rosenstiel Basic Medical Sciences Research centre, Brandeis University, Waltham, USA

16.00

Dr. Paul BUNDOCK

" *Agrobacterium tumefaciens* T-DNA: a natural tool for gene targeting in plants, yeasts and filamentous fungi "

Institute of Plant Sciences, Leiden University, The Netherlands

17.00

Social gathering and drinks

19.00

Dinner

20.30

Dr. Roman ULM

"Genotoxic stress and homologous recombination in *Arabidopsis thaliana"*

Friedrich Miescher Institute, Basel, Switzerland.

Friday June 30th

07.00

Breakfast

8.00

Pr. Jürg KOHLI

"Meiosis and recombination hotspots in fission yeast"

Institute of General Microbiology, University of Bern, Switzerland

9.00

Dr. Joska ZAKANY

"Homologous recombination and functional genomics in mouse ES cells"

Dept de zoologie, Sciences III, UNI Genève, Switzerland

10.00

Coffee break

10.30

Dr. Didier SCHAEFER

"Homologous recombination and functional genomics in *Physcomitrella patens"*

Institute of Ecology, Plant Cell Genetics, UNILausanne

11.30

Closing remarks

End of the symposium and lunch

Genetic Requirements of Double-Strand Break Induced Recombination in *S. cerevisiae*.

Neal Sugawara, Eli Kraus, Grzegorz Ira and James Haber.

Rosenstiel Center, Brandeis University, Waltham, MA 02454-9110 USA.

Our work utilizes the HO endonuclease of *S. cerevisiae* to induce a double strand break (DSB) *in vivo* and we then monitor how the DSB is repaired via homologous recombination or by non-homologous end joining. An overview will be presented that reviews the genetic requirements of several DSB-induced processes that we can monitor. These include the generation of the single stranded tails, gene conversion and single strand annealing, the removal of non-homologous tails from recombination intermediates, mismatch repair and non-homologous end joining. Understanding the genetic requirements for DSB-induced recombination and non-homologous end-joining may prove useful in understanding the genetic requirements for gene insertions by homologous or non-homologous recombination. To explore this we developed an assay where DNA transformed into yeast can use either homologous recombination or a non-homologous insertion pathway to integrate into the genome. This assay is being used to determine which genes are important for integration via the non-homologous integration pathway.

Homeologous recombination, horizontal gene transfer and bacterial evolution

Ivan Matic and Miroslav Radman

E9916 INSERM, Faculte de Medecine-"Necker - Enfants malades", Universite Rene Descartes-Paris V, Paris, France

Genomic DNA sequence polymorphism is the structural element of the genetic barrier between different enterobacterial species and strains in conjugational and transductional crosses. The efficiency of the genetic barrier, which is barrier to genetic recombination, is determined by the efficiency of recombination (principally RecA) and mismatch repair (principally MutS and MutL) proteins. The induction of the SOS response during interspecies conjugation is proportional to the genomic sequence divergence of the two mating partners. A quantitative analysis relating recombination frequencies in conjugational crosses and genomic sequence divergence leads to the following conclusions: (1) There is a log-linear relationship between recombination and sequence divergence, (2) Mismatch repair affects the slope of this log-linear relationship: overexpression of the MutS and MutL proteins greatly restricts horizontal gene exchange, whereas the loss of these functions reestablishes the interspecies gene exchange, (3) SOS induction, or the overexpression of the RecA protein, changes the intercept, not the slope, of the recombination-divergence relationship. This powerful control of the horizontal gene transfer suggests that environmental and physiological factors affecting mismatch and SOS repair systems also affect the efficiency of genetic barriers and, consequently, the rate of evolution of bacterial species.

Agrobacterium tumefaciens T-DNA: a natural tool for gene targeting in plants, yeasts and filamentous fungi.

Paul Bundock, Marcel J.A. de Groot*, Amke den Dulk-Ras, Haico van Attikum, Alice G.M.Beijersbergen*, Aaron A. Winkler, Yde H. Steensma & Paul J.J. Hooykaas. Institute of Molecular Plant Sciences, Clusius Laboratory, Leiden University, Wassenaarseweg64, 2333 AL, Leiden, The Netherlands.

* Unilever Research Laboratory, Oliver van Noortlaan 120, 3133 AT, Vlaardingen, The Netherlands.

Agrobacterium tumefaciens is a gram negative soil bacterium and the causative agent of crown gall disease. The bacterium induces the formation of crown galls or tumours at plant wound sites. During tumorigenesis, *A. tumefaciens* transfers a part of its tumour inducing (Ti) plasmid, the T-DNA, to the plant cell where it then integrates into the plant genome at a random position. Due to the ease of use, the high transformation frequencies and the simple integration patterns obtained when using *Agrobacterium*, it has remained the most popular method for plant transformation.

The efficiency of T-DNA integration makes it a good substrate for gene targeting experiments. Several approaches have been designed to study gene targeting in *Arabidopsis thaliana* and *Nicotiana* sp. and these will be discussed.

A. tumefaciens is also able to transfer T-DNA to the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. We have studied the effect of these alternative hosts on the mechanism of T-DNA integration. When a T-DNA carrying sequences homologous to the yeast genome was transferred to *S. cerevisiae* it integrated efficiently via homologous recombination. This demonstrates that the mechanism of T-DNA integration is determined by the host cell, and not by the T-DNA itself and/or the accompanying Vir proteins. Furthermore, a T-DNA lacking homology with the *S. cerevisiae* genome can integrate via illegitimate recombination. Gene targeting is less efficient in other fungi. In *K. lactis*, a commercially important yeast strain, the majority of transformants contain the introduced DNA integrated randomly into the *K. lactis* genome. We have shown that gene targeting in *K. lactis* can be dramatically improved when T-DNA rather than double stranded naked DNA is used as a recombination substrate.

A.tumefaciens can also transfer T-DNA to the filamentous fungus *Aspergillus awamori*, demonstrating for the first time DNA transfer between a prokaryote and a fungus. Gene targeting experiments in *Aspergillus* have been successful and this opens up new possibilities for the study of gene function in filamentous fungi.

Genotoxic Stress and Homologous Recombination in *Arabidopsis thaliana*

Roman Ulm, Moez Hanin, Tesfaye Mengiste, Katya Revenkova, Zerihun Tadele and Jurek Paszkowski, Friedrich Miescher Institute (FMI), Basel

Gene targeting in plants was first achieved at low frequency in 1988. To date the low frequency of precise engineering of the plant genome still holds true (except for moss). To identify components possibly necessary for the process of homologous recombination, a genetic approach was undertaken.

We performed a genetic screen of a T-DNA tagged *Arabidopsis* mutant collection for hypersensitivity to the DNA damaging agent methyl methanesulfonate (MMS). In this screen, we identified several mutants impaired in response to different genotoxic stresses (MMS, UV-C, X-rays). Two of these mutants will be presented in more detail.

The *mim* mutant (hypersensitive to *M*MS, *i*rradiation and *M*itomycin C) is deficient in an SMC-like (*s*tructural *m*aintenance of *c*hromosomes) component, with Rad18 of fission yeast as its closest relative. *mim* was shown in an intrachromosomal recombination assay to have about 4-fold reduced recombination properties.

Another mutant, *mkp1*, is impaired in a component involved in the regulation of genotoxic stress signalling. Interestingly, the mutant is also altered in its response to the plant stress hormone abscisic acid (ABA) indicating that it is affected in a shared component between the two signal transducing pathways.

Meiosis and recombination hotspots in fission yeast

Jürg Kohli, M. Baur, A. Grishchuk, E. Lehmann, M. Molnar, P. Munz, S. Parisi, Institute of General Microbiology, University of Bern, juerg.kohli@imb.unibe. ch

In sexually reproducing eukaryotes specifically differentiated diploid cells undergo meiosis to produce four haploid cells after one round of DNA replication and two divisions. Recombination (crossover formation) is required for proper chromosome segregation in the meiotic divisions. Special features of meiosis in S. pombe are the concomitant absence of a tripartite synaptonemal complex and of crossover interference, and the maintenance of the bouquet structure of chromosomes throughout prophase I (1). Based on classical genetics carried out in the sixties the M26 hotspot of recombination was studied. It is created by the single base substitution mutation ade6-M26. Changes in chromatin structure associated with the M26 hotspot were demonstrated (2). Recently the new hostspot ura4-aim was analysed.

One project is dedicated to the analysis of proteins involved in meiotic recombination and other meiotic processes. rec8 mutants are defective in recombination in a region-specific way, in sister chromatid cohesion, in linear element formation, in chromosome pairing, and in segregation of the chromosomes in the meiotic divisions. As a result, spore viability is low. The clustering of telomeres and centromeres (bouquet formation) is not affected. Rec8p is phosphorylated during prophase I and persists beyond the first meiotic division. Genes homologous to rec8 (cohesins) are present in all eukaryotic cells (3, 4). Currently we are completing the initial characterisation of the early recombination genes rec7 and rec15. Mutation of these genes leads to reduction of recombination throughout the genome, and to other phenotypes that differ from the rec8 mutation phenotypes. Future work is aimed at the identification of interactions of Rec8p with Rec7p, Rec15p and other meiotic proteins, in particular with respect to initiation of recombination (see below).

The M26 hotspot of recombination is currently studied in several laboratories. We published that the ura4-aim hotspot (an artificially introduced marker gene) located 15 kb from ade6 interacts with the M26 hotspot: Presence of both hotspots in the same cross leads to reduction of recombination frequency at both hotspots (5). We are now completing the analysis of the the ura4-aim hotspot. In the future we plan to use both hotspots for the study of the initiation of meiotic recombination by double-strand break formation (6).

1) Kohli, J. 1994. Curr. Biol. 4: 724-727
2) Mizuno, K-i. Et al. 1997. Genes & Dev. 11: 876-886
3) Molnar, M. et al. 1995. Genetics 141: 61-73
4) Parisi, S. et al. 1999. Mol. Cell. Biol. 19: 3515-3528
5) Zahn-Zabal, M. et al. 1995. 140: 469-478
6) Cervantes, M. D. et al. 2000 Mol. Cell in press

“Homologous recombination and functional genomics in mice”

Joszef Zakany, Department of Zoology and Animal Biology, University of Geneva, Zakany@zoo.unige.ch

The objective of this presentation is to illustrate how targeted genome manipulations inside a gene cluster were made possible by homologous and site specific recombination.

Targeted mutagenesis through disruption, or replacement of the coding part of a gene occurs in the nucleus by homologous recombination between the genomic DNA and an appropriately designed targeting vector. Targeting vectors are prepared as plasmids, through standard molecular biological subcloning techniques, and contain a strategically placed selection cassette flanked by, typically, several kilobases of homologous genomic DNA, which are the targets of "legitimate" intramolecular recombination events. In standard protocols, the targeting vector is transfected into embryonic stem (ES) cells by electroporation, and transformants are selected *in vitro* on drugs appropriate for the selection cassette, like *neomycin* or *hygromycin*. Drug resistant clones are further screened to find the homologous recombinants involving, eventually, Southern blots. Both 5' and 3' restriction enzyme recognition sequences and genomic probes used in this screen should lie outside of the extent of the targeting vector. Such alleles of a gene are called knock-out, or null alleles, and were the first types of targeted alleles. Today targeted allele variants include different forms of knock-in alleles. Most current designs also involve the *loxP*/Cre system of bacteriophage *P1*. Such alleles allow sequential modifications to the targeted locus. Site specific recombination is induced by the *Cre* recombinase between two knocked-in *loxP* sites located, in *cis* or in *trans*. Subtle mutations, when the selection cassette is ultimately removed from the locus, targeted deletions, inversions, duplications can be aimed at this way.

ES clones that carry the desired allele are introduced into early pre-implantation stage embryos, typically blastocysts, to form chimeric mice. Upon breeding, a fraction of the chimeras passes the mutant allele on to the F1 generation, allowing establishment of mutant mouse lines. Further refinements in targeting vector design and the recently exploding number of Cre expressing mouse lines give rise to ever increasing possibilities of raising conditional alleles, to facilitate the analysis of various aspects of lethal pleitropic mutations. Recent variants of the *loxP* nucleotide sequence increase further the sophistication of allele design also facilitating targeted transgene insertions.

Establishment of issuing mutant mouse stocks proved invaluable in functional analysis of the *HoxD* cluster, a multigene complex of developmental regulatory genes, about 100kbps in size.

Conventional gene knock-out alleles of all the genes of the *HoxD* cluster were raised, starting with *Hoxd13*, providing genetic proof of the role of this gene cluster in spatial and temporal control of mammalian embryonic development. A new generation

lacZ knock-in allele of *Hoxd1* brought into focus the role of mRNA stability, in addition to more conventionally observed modes of transcriptional control.

Targeted modifications of regulatory regions led to modulation of neighbor genes' expression. The importance of timing in gene function was established.

Gene transpositions revealed instances of global regulatory interactions. Several regulatory regions were found to serve delaying activation of late-expressed, posterior genes.

Overlapping deletions uncovered redundant roles of nearby genes. Sets of neighbor genes were found to be subject to distantly located global enhancers. The importance of gene dose effects in regulation of patterning by *HoxA* and *HoxD* genes was established.

loxP sites present at various locations in the *HoxD* gene complex and *Cre* recombinase specifically expressed in spermatocytes allowed the production of a large number of recombinant alleles through targeted meiotic recombination. Translocations, deletions and duplications were produced in mice, and their effects are being analyzed.

Targeted inversions were produced by *loxP*/Cre mediated recombination, and start to yield insights into molecular mechanism that lie behind broad regulatory phenomena characteristic of the *Hox* complexes, like spatial and temporal colinearity.

Homologous recombination and functional genomics in the moss *Physcomitrella patens*

Didier Schaefer*, S. Vlach*, M. Chakhaparonian*, J.-P. Zrÿd*, N. Houba-Hérin**, K. Von Schwartzenberg**, C. Pethe** et M. Laloue**, (*) Institut d'écologie-phytogénétique cellulaire, Université de Lausanne, (**) Laboratoire de génétique cellulaire, INRA Versailles

The tremendous amount of DNA sequence information from all types of living organisms obtained in the last decade generates a real revolution in biology. The next challenge is now to understand the function of all these genes and is referred as functional genomics. Global functional genomic approaches are conducted using DNA microarrays and this allows the establishment of the expression profile of many if not all the genes of an organism in response to different experimental conditions. Stochastic functional genomic approaches are based on the generation of a collection of insertional mutants by the random integration in the genome of identified tags such as transposons or T-DNAs. The development and the extensive use of T-DNA tagged libraries in the model plant *Arabidopsis thaliana* is an example of the power of such approach.

However, the most powerful tool to analyse gene function is to generate mutations in the gene by homologous recombination mediated transformation (gene targeting). The main factor allowing the realisation of this type of experiments is the ratio of targeted to random integration events observed upon genetic transformation. This ratio varies a lot in living cells, being close to 100 % in bacteria, ranging from 1-100% in yeast and in some filamentous fungi and protozoa, and falling down to 0.1% to 0.001% in higher eukaryotes such as plants and animals. In 1989, the observation that mouse embryonic stem cells (ES cells) have a ratio of targeted to random integration events ranging from 0.1 to 10% has enabled the generation of specific mutations in the mouse genome, leading to the development of the mouse ES cell model system that is broadly used now a days. In 1997, we have demonstrated that the ratio of targeted to random integration events in the moss *Physcomitrella patens* is around 90%, opening the way for the first time in plant to fine functional genetic analyses. We shall present experiments conducted to characterise gene targeting events and to further develop functional genomic approaches in this moss.

A detailed analysis of gene targeting features in *Physcomitrella* was conducted on the adenine phosphoribosyl transferase (*apt*), taking advantage of the fact that loss of *apt* function generates plants resistant to the adenine analogue 2,6-diaminopurine (DAP). Using a cDNA based replacement vector, replacement of the *apt* locus was successfully achieved by a double homologous recombination event occurring within stretches of homologous sequences as short as 52 and 187 bp, although at low efficiency. Homologous recombination within such short stretches of homology are at the limit of feasibility of gene conversion in *S.cerevisiae*. Targeted insertion of the transforming vector mediated by a single homologous recombination event associated with an end joining reaction between vector and genomic sequences was also observed. The presence of short stretches of heterologous sequences at the extremities of the transforming DNA

was shown to impair conversion events and a detailed analysis of insertion events indicated that homologous recombination was achieved in a DNA synthesis-dependent process. Using genomic DNA based replacement vectors, we have been able to show that 2 stretches of homologous sequences around 500 bp were sufficient to saturate the efficiency of gene targeting in the moss genome. With such gDNA based vectors, targeted integration in the *apt* locus was ca 100 fold more frequent than random integration, and gene conversion by double cross-over were observed at similar frequencies than targeted insertion by single cross-over. Thus the powerful functional genomic approaches developed in yeast can be directly applied to study gene function in *Physcomitrella patens* with the same efficiency.

The specificity of gene targeting was addressed on a specific member of the chlorophyl a/b binding protein (*cab*) multigene family. The *cab* multigene family of *Physcomitrella* is composed of at least 15 members sharing ca 90% sequence homology at the nucleotide level. Using an insertion vector carrying 1 kb of the *cab* gene ZLAB1, targeted insertion in that gene was achieved in 30% of transformed strains, providing evidence for the high specificity of gene targeting in *Physcomitrella*.

A further refinement in the use of efficient gene targeting is to combine it with the use of site specific recombinase such as the Cre/lox system to induce further specific mutations in the genome. Transient expression of a 35S-Cre cassette was performed on protoplasts of a transgenic moss strain carrying a single copy of a 35S-neo marker flanked by two lox sites. Neomycin sensitive clones were recovered at frequencies in the range of the percentage, and molecular analysis of these strains confirmed that the neo marker was correctly excised by the Cre recombinase. Thus, the Cre/lox system can be used in *Physcomitrella* like in mouse ES cells to induce specific modification in the genome and recycle selectable markers.

Finally, the development of a new model system to study the function of plant genes requires that the knowledge obtained in *Physcomitrella* is relevant to higher plant biology. Unexpectedly, *apt*- moss strains displayed a strong developmental phenotype in addition to DAP resistance, and we took advantage of this observation to test the above assumption. To do this, *apt*- strains of *Physcomitrella* patens were transformed with the corresponding At*apt*1 or At*apt*2 genes of *Arabidopsis* cloned under the control of the rice actin-1 gene promoter. Complementation of the *apt*- phenotype was observed with each *Arabidopsis* gene demonstrating that higher plant genes are functional in moss and that the functional information obtained in moss is relevant to higher plant biology.

The moss *Physcomitrella patens* provides an outstanding and so far unique model system that advantageously complements the tools available in *Arabidopsis thaliana* for plant functional genomics. It also provides a system to study the genetic regulation of the ratio of targeted to random integration events which may enable the development of new strategies to target genes in higher plant, as well as in other organisms.

List of participants

PhD students

NAME	UNIVERSITY	E-MAIL ADDRESS
ALMERAS Emmanuelle BELLAFIORE Luanne	IE-BPV UNI LAUSANNE Plant Biology, UNI GENEVE	Emmanuelle.almeras@ie-bpv.unil.ch
BELLAFIORE Stéphane BOERNER Tim	Plant Biology, UNI GENEVE Plant Physiology UNI BERN	Stephane.bellafiore@molbio.unige.ch Tim.boerner@pfp.unibe.ch
CHAKHAPARONIAN Micha CHRISTINET Laurent	IE-PC, UNI LAUSANNE IE-PC, UNI LAUSANNE	Mikhail.chakhparonian@ie-pc.unil.ch Laurent.christinet@ie-pc.unil.ch
CLUZET Stéphanie EGGMANN Thommas	IE-BPV, UNI LAUSANNE Botanique UNI NEUCHATEL	Stephanie.cluzet@ie-bpv.unil.ch Thomas.eggmann@bota.unine.ch
FINKA Andrija FISCHER Urs	IE-PC, UNI LAUSANNE Plant Physiology UNI BERN	andrija.finka@ie-pc.unil.ch Urs.fischer@pfp.unibe.ch
KURSTEINER Oliver LANGLADE Nicolas	Plant Physiology UNI BERN Botanique UNI NEUCHATEL	Oliver.kuersteiner@pfp.unibe.ch Nicolas.langlade@bota.unine.ch
LENTZE Nicolas LIECHTI Robin	Plant Physiology UNI BERN IE-BPV UNI LAUSANNE	Nicolas.lentze@pfp.unibe.ch Robin.liechti@ie-bpv.unil.ch
PROUSSACOVA Julie ROTH Christian	IE-PC UNI LAUSANNE IE-BPV UNI LAUSANNE	Julie.prussacova@ie-pc.unil.ch Christian.roth@ie-bpv.unil.ch
SCHIPPER Ori SCHUMPP Olivier	Plant Sciences, ETH ZURICH Institute of Botany UNI BERN	Ori.schipper@ipw.biol.ethz.ch Schumpp@pfp.unibe.ch
SUTER Karin VOGLER Hannes	Plant Physiology UNI BERN Plant Physiology UNI BERN	Karin.suter@pfp.unibe.ch Hannes.vogler@pfp.unibe.ch

Post docs

BARIOLA Pauline	IE-BPV UNI LAUSANNE	Pauline.bariola@ie-bpv.unil.ch
BUCKA Alexander	LAU UNI LAUSANNE	Alexander.bucka@lau.unil.ch
FUTTERER Johannes	Plant Sciences ETH ZURICH	Johannes.fuetterer@ipw.biol.ethz.ch
GONNEAU Martine	Biologie Cellulaire INRA VERSAILLES	Gonneau@versailles.inra.fr
HINZ Ursula	IE-PC, UNI LAUSANNE	Ursula.hinz@ie-pc.unil.ch
IAVICOLI Anna-Lisa	UNI FRIBOURG	annalisa.iavicoli@unifr.ch
PERROUD Pierre-François	IE-PC, UNI LAUSANNE	pierre-francois.perroud@ie-pc.unil.ch
BOURNIQUEL Aude	NESTLE Epalinges	aude.bourniquel@rdls.nestle.com
UZE Murielle	IE-PC, UNI LAUSANNE	Murielle.uze@ie-pc.unil.ch

Participants of GT 2000 and MOSS 2000

KNIGHT Celia	UNI LEEDS	gen6ck@south-01.novell.leeds.ac.uk
PANVISAVAS Nathinee	UNI LEEDS	Bgynp@leeds.ac.uk
HASEBE Mitsuyasu	NIBB Okasaki, Japan	Mhasebe@nibb.ac.jp
FUJITA Tomomichi	NIBB Okasaki, Japan	Tfujita@nibb.ac.jp
SAKAKIBARA Keiko	NIBB Okasaki, Japan	Bara@nibb.ac.jp
NISHIYAMA Tomoaki	NIBB Okasaki, Japan	Tomoaki@nibb.ac.jp
HIWATASHI Yuji	NIBB Okasaki, Japan	Hiwatash@nibb.ac.jp
SHIN Jeong-Sheop	Korea University	Jsshin@mail.korea.ac.kr
CARNEIRO Patricia	Biologie Washington	carneiro@biology.wustl.edu
KOPRIVOVA Anna	AG Reski, UNI FREIBURG	Koprivov@uni-freiburg.de

VON ORLOW Melanie	Freie Universität Brelin	Annine@chemie.fu-berlin.de
PRICE Michelle	Lab Bioenergetics, UNI GENEVE	Mprice@lehmann.mobot.org
CLARK Alex	Lab Bioenergetics, UNI GENEVE	Clark@bioen.unige.ch
COVE David	UNI LEEDS	D.J.Cove@leeds.ac.uk
CUMING Andrew	UNI LEEDS	a.c.cuming@leeds.ac.uk
WOOD Andrew	Plant Biology, Southern Illinois University	Wood@plant.siu.edu
QUATRANO Ralph	Biologie Washington	Rsq@biology.wustl.edu

Guest Lecturers

BUNDOCK Paul	Plant Science, Leiden University	Bundock@rulbim.leidenuniv.nl
KOHLI Jürg	Microbiology, Univeristy of Bern	Juerg.kohli@imb.unibe.ch
MATIC Ivan	Necker, Univerity of Paris V	Matic@necker.fr
SUGAWARA Neal	Rosenstiel center, Brandeis University	Sugawara@hydra.rose.brandeis.edu
ULM Roman	Friedrich Miescher Institute, Basel	Ulm@fmi.ch
ZAKANY Joska	Zoology, University of Geneva	Jozsefzakany@zoo.unige.ch

Organizers

SCHAEFER Didier	IE-PC, University of Lausanne	Didier.schaefer@ie.pc.unil.ch
ZRYD Jean-Pierre	IE-PC, University of Lausanne	Jean-pierre.zryd@ie-pc.unil.ch