Report of the activities carried out by Dr Francesco Paolocci at Institute of Integrative Biology of the Cell (I2BC) Université Paris-Sud - host scientist Prof. Fabienne Malagnac

The proposed STM program aimed at reaching three distinct, although related, goals:

1) Identification and characterization of the mating type genes (*MAT*) in *Ascobolus immersus*;
2) Comparison of the structure and organization of the *MAT* idiomorphs in the *Pezizomycetes Ascobolus* and *Tuber* spp;
3) Assessment of *A. immersus* as a model species for functional genomics of truffles, symbiotic Ascomycetes belonging to the *Tuber* genus.

To reach goal 1, the genomic regions flanking the *MAT1-1-1* gene, already identified *in silico* in the sequenced genome of *A. immersus* strain RN42 (unpublished data), has been used as template for identifying by positional cloning the alternative mating type gene (*MAT1-2-1*) in strains sexually compatible with it. To this purpose, DNA has been isolated from 4 strains that cross with RN42; then primers have been designed on genes flanking the mating type gene in RN42 to search by PCR for their orthologs in MAT+ strains. The rationale behind the selected approach was that genes around the MAT idiomorphs are conserved among strains of different mating types in all *Pezizomycetes* tested thus far (Rubini et al. 2011; Belfiori et al. 2013). Thus, successive rounds of PCR to move away, both upstream and downstream of the *MAT1-1-1* gene have been performed to identify genes conserved between *MAT+* and *MAT*– strains. In total, primer pairs relative to 18 genes, 9 upstream and 9 downstream of the *MAT1-1-1* gene in RN42 have been designed and tested. The genomic region scanned by PCR spanned more than 250 Kb in *MAT* – strains, about 190 Kb upstream and 80 Kb downstream the *MAT1-1-1* gene. All the 18 primers pairs yielded the expected amplicons on RN42 reference strain as well as on other strains harboring the *MAT1-1-1* gene. Conversely, the first eight upstream and the first 7 MAT1-1-1 downstream primers failed to yield the expected amplicons in any MAT+ strain tested, regardless of their origin. Only the most upstream (about 180 Kb) and downstream (about 72 kb) primer pairs produced the expected amplicons also on MAT+ strains. Overall, present results suggest two alternative explanations: a) the idiomorphic regions in *Ascobolus* are much larger than in other *Pezizomycetes* tested thus far and they might contain several genes specific to each mating type; b) the genomic region surrounding the *MAT1-2-1* gene is highly compacted in *MAT+ Ascobolus immersus* strains, likely because of the presence of GC rich regions that might prevent correct DNA amplification. Indeed, the use of DNA polymerases and PCR buffers optimized for the amplification of long amplicons and GC rich regions did not allow us to overcome this problem. Therefore, regardless of the reason, it has been conveyed to sequence the genome of a strain sexually compatible with RN42 to identity and characterize the alternative *MAT* gene in this species.

To reach goal 2, the mating type genes and *MAT* locus identified in *Ascobolus* and *Tuber* spp have been compared using different genomic tools and programs. Notably, beside the region coding for the *alpha* box domain the extent of similarity between the *MAT1-1-1* gene from *Ascolobus* and *Tuber* spp is low. The organization of these genes also differs for the position of the single intron. As far as it concerns the genomic regions surrounding the *MAT* locus no synteny has emerged when the scaffolds containing the *MAT* locus from different *Tuber* spp (i.e *T.melanosporum*, *T. aestivum* and *T. magnatum*) were compared with that from *Ascobolus*. In the latter species linked to this locus is the *SLA2* gene as well as it occurs in many other *Ascomycetes* but not in *Tuber* spp. Linked to the *MAT* locus of *Tuber* spp are conversely present ORFs never found next to this locus in *Ascobolus*. Overall, present investigation has shown that gene content next to the *MAT* locus differs between *Ascobolus* and *Tuber* spp.

In stark contrast to *Tuber* spp., *Ascobolus immersus* can be easily cultivated and mate under controlled conditions to complete its life cycle in about 2 weeks. Additionally, this species is prone to direct gene transfer into its protoplasts via polyethylene glycol treatment. Because of these positive traits, the third goal of present project was to use *Ascobolus* as model organism to gain functional evidence on regulatory regions of sex genes from truffle species. To this end, the 1 kb long region upstream the start codon of the *MAT1-1-1* and *MAT1-2-1* genes and, as control, of *GAPDH*, were PCR amplified from genomic DNA isolated from *T.melanosporum* mycelial strains of opposite mating type using a Taq DNA polymerase endowed with proof reading activity. The resulting amplicons were sequenced to confirm their identity as well as the absence of mutations; then each promoter region was cloned in front of eGFP reporter gene by means of ligation into a *Sma*I pre-linearized pBC-hygro vector. Beside eGFP, this vector presents the cassette for hygromycin resistance. The three final vectors, each containing one truffle promoter region in front of eGFP, were then used to transform *Ascobolus immersus*. From each transformation several hygromycin resistance individual strains were obtained. DNA were isolated from these putatively transgenic lines and PCR amplified to test for the presence of expected promoter. The positive PCR amplification confirmed the presence of each truffle promoter into *Ascobolus immersus* transformants.

As follow up of this project the host Institution will cross at least one transgenic line *per* transformation to a wild type strain to collect spores and select, on a selective medium, homokaryotic transgenic lines that will be supplied to Francesco Paolocci to monitor in his lab the transgenic strains for GFP expression.

Finally, during this stage a proposal in collaboration with Dr Claude Murat (UMR 1136 Interactions Arbres-Microorganisms) focused on *Ascobolus* as model system for functional genomics of *Tuber* spp has been submitted by Prof F Malagnac and Dr F. Paolocci to the Labex Arbre Funding agency (call for Proposal 2016-Innovative Projects in Research and Translational Research).