Project no.: **LSHB-CT-2004-511952**

Project acronym: FUNGWALL

"The FUNgal cell WALL as a target for antifungal therapies"

Instrument: Specific Targeted Research Project

Thematic Priority: Life Sciences, Genomics and Biotechnology for Health

Publishable Final Activity Report

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Project coordinator: Jean-Paul LATGE
Project coordinator organisation: Institut Pasteur, Paris (France)

Table of Contents

Section 1 – Project execution 3
Summary description of project objectives 3
Contractors involved 4
Work performed and end results 4
Achievements of the project as regards the state-of-the-art 5
Impact of the project on its industry or research sector 5
Section 2 – Dissemination and use 6

Section 1 – Project execution

Summary description of project objectives

The cell wall of pathogenic fungi is a good target for the development of new drugs for the following reasons: (1) The fungal cell wall is required for fungal cell integrity and is essential for fungal growth and for virulence; (2) Polysaccharidic components of the cell wall are unique to fungi and consequently, putative inhibitors of the biosynthetic pathways responsible for cell wall construction can be potent antifungals, as shown by the recent launch by big Pharmas of drugs inhibiting ß1-3 glucan synthesis.

The STREP FUNGWALL objectives are centered on the assembly of the cell wall polysaccharide skeleton. The enzymes and reactions associated with chitin, β glucan and mannan synthesis, β glucan cross-linking and branching will be investigated. New post genomic approaches will enable us to first revisit old targets, define novel targets and to screen for novel compounds that disrupt the integrity of the cell wall with the goal of identifying new generation antifungals that target fungal cell wall biosynthesis. These studies will use primarily the 2 main fungal pathogens in Europe, *Candida albicans* and *Aspergillus fumigatus.*

To identify cell wall targets, 4 work packages have been defined:

- **WP1** studies **chitin**; in WP1, the assembly and modelling of the cell wall chitin will be revisited using a multidisciplinary approach to develop and validate new inhibition assays for the discovery of the first chitin synthesis and chitinase inhibitors to be efficient in vivo against fungal cells.
- **WP2** will revisit **ß1-3 glucan** synthesis and propose a new post genomic approach to better understand the inhibitory role of echinocandins; in addition, this WP will investigate for the first time endo ß1-3 glucanase as a putative target.
- **Branching and cross-linking between chitin and ß1-3 glucan** is required in the formation of a resistant skeleton of the cell wall. Linkages between these 2 polysaccharides have been shown to be essential to construct the fibrillar core of the fungal cell wall. Accordingly, enzymes and regulators involved in the biosynthesis of this core structure will be key target molecules. At this time the nature of the enzymes that are responsible for the branching and cross-linking of the structural cell wall polysaccharides. Their identification will be a major research objective of this application and will form the **WP3** of our STREP.
- Sensing wall modifications and damage and restoring cell wall integrity require a functional network of active sensors and signalling mechanisms. **O-mannosylation** plays an essential role in maintaining the integrity of these sensing mechanisms. **WP4** will study mannosylation in fungi with the objective to identify essential pathways and proteins that become inactive in absence of post translational mannosylation.

FUNGWALL

Contractors involved

Partner	Participant name	Organisation	Country	Scientific Leader
1	Institut Pasteur, Paris	IP	FR	Jean-Paul LATGE
2	University of Aberdeen	UNIABDN	GB	Neil GOW
3	University of Amsterdam	UVA	NL	Frans KLIS
4	INSA Toulouse	INSA	FR	Jean-Marie FRANCOIS
5	CNRS Marseille	CNRS	FR	Bernard HENRISSAT
6	University of Salamanca	USAL	ES	Carlos R. VASQUEZ
7	University of Heidelberg	UHEI	DE	Sabine STRAHL
8	University Complutense of Madrid	UCM	ES	Maria MOLINA
9	University of Dundee	UNIVDUN	GB	Dan VAN AALTEN
10	Novexel Romainville	NOE	FR	Michael BLACK

Table 1: List of FUNGWALL partners

The FUNGWALL Consortium is coordinated by Prof. Jean-Paul LATGE of Institut Pasteur, Paris (Partner 1).

Work performed and end results

Significant progress has been made in all the WPs presented. Over the 3-year period, 75% of the deliverables were completed. Two reasons can be put forward to explain the lack of completion of 100% of the deliverables. First, for some of the deliverables, the impossibility to complete them was due to technological issues. For the other deliverables, the full success has inevitably been affected by the failure of the European Commission to grant a no-cost extension that would have allowed several of the partners to work for a true 36-month term for the project.

Major achievements accomplished during this grant are the following:

(1) The validity of chitin synthesis as a legitimate target for antifungal chemotherapy was underlined by the discovery of strong synergistic combinations of anti-chitin and anti-glucan inhibitors.

(2) Chitin hydrolysis was validated as target for antifungal chemotherapy and novel inhibitors of these enzymes were discovered and characterised following crystallisation of various chitinases.

(3) Complete mutant collections of all chitin synthase genes was achieved and characterised for the two major human pathogens *Candida albicans* and *Aspergillus fumigatus* and has allowed for the development of new screens for chitin synthase inhibitors.

(4) The mode of action of aminocandin was elucidated though various genomic strategies.

(5) A new β1,3 glucan binding domain has been characterized and shown to be essential in yeast morphogenesis.

(6) Two new transglycosidase activities have been discovered and shown to be involved in branching of β1,3 glucans and the synthesis of β1,6 glucans.

(7) A list of genes with putative cell wall polysaccharide remodeling activity has been identified bioinformatically and validated through various genomic and biochemical approaches.

(8) Several methodologies to analyse carbohydrate-protein interactions have been developed.

(9) The signals determining 0-mannosylation have been identified.

(10) A list of all O-mannosylated proteins of *S. cerevisiae* was established and their role in sensing the environment analysed.

(11) The essentiality of O-mannosylation in yeasts and moulds was underlined.

(12) Two major international conferences on cell wall have been organized.

Achievements of the project as regards the state-of-the-art

Project achievements have placed Europe in a leading position in the world for the analysis of fungal cell wall. The coupling of biochemical and genetical methodologies was extremely synergetic to tackle this problem and have given a unique flavour to our STREP. A close contract between the different members of the STREP will now continue and will lead to new scientific developments in the area. We hope that this collaboration will continue to be supported by Europe.

Impact of the project on its industry or research sector

Major achievements of the STREP FUNGWALL have resulted from the new multidisciplinary genomic and post genomic approaches undertaken in this STREP. Among them we can site the following:

1. *A.fumigatus* arrays have been produced in view to analyse combination of drugs.
2. Molecular characterization of fungal endo β1,3 glucanase activity has led to a screen for glucanase inhibitors.
3. Synthetically lethal technology has been applied to transglycosidase candidates and identified new interactive partners.
4. Methodologies for biochemical HTS of transglycosidase have been developed.
5. A comprehensive list of cell wall proteins has been produced and analysed in silico and *in vivo.*

Several new drug targets have been identified during the course of this STREP. Among them:

- chitinases and endo β1,3 glucanases;
- new transglycosidases remodelling β glucans;
- O-mannosyltransferases.

Most strains have been engineered which could be very useful tools in the search for new drugs.
For example, chitin synthase mutants are now available for looking for specific antifungal drungs in both *Candida* and *Aspergillus*.
Although we have identified new drug targets and revisited successfully old ones, we have assisted during the course of this STREP to a reduction in the research and developments efforts put by the pharmaceutical industry in the antifungal business. The chances to see new drugs developed by these companies in the near future are very limited. To palliate this, we have also embarked in a combination therapy strategy that has been based on the research of this STREP on fungal cell wall biosynthesis. It will lead to promising results in the management of patients in Europe suffering from systematic fungal infections. This may lead to new formulation of existing antifungal drugs, a very promising development for SMES.

Section 2 – Dissemination and use

Fungal pathogens represent the major eukaryotic agents of serious infection in European countries. Infections due to *Candida albicans* and *Aspergillus fumigatus* are the most common and clinically important pathogens and are therefore the focus of this project. The repertoire of available antifungal chemotherapeutic agents is inadequate to treat life-threatening infections. Therefore there is an urgent need to generate more efficacious antifungal compounds than the ones available. We have focused our programme on the core cell wall structure that is common to all fungal pathogens and is a major drug target because it is essential for their growth and viability. This core consists of the component polysaccharides 1-3 glucan and chitin, which are cross-linked to each other. Major achievements have been obtained. Among them:
(1) the analysis of chitin and glucan synthase as drug targets and the discovery of strong synergistic combinations of anti-chitin and anti-glucan inhibitors;
(2) the validation of glycosylhydrolases such as chitinases as target for antifungal chemotherapy;
(3) the discovery of two new transglycosidase activities;
(4) the confirmation of the essentiality of O-mannosylation in yeasts and moulds.

Two major international conferences on cell wall have been organized during the project's lifetime.

Many drug targets amenable to HTS for the identification of new families of drugs have been identified or confirmed. There is now room for industry to follow up the applied results obtained in our STREP.

A major advantage of the STREP has been to start a true European research in fungal cell wall by forcing European scientists to work together in the area. This collaboration will continue in the future. Hopefully the worldwide leadership of the fungal cell wall science will remain in Europe.