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Response and tolerance to stress: the power of the analyses at the genome and the microbial system levels

Resposta e tolerância a stresse: o poder das análises à escala do genoma e do sistema microbiano

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Isabel Sá-Correia[1]

ABSTRACT

This communication addresses a topic of paramount importance in Biology and Biotechnology: the microbial responses and tolerance to environmental stresses, at a systems level. The focus is on the contemporary approach of Microbial Physiological Genomics that is proving to be vital in providing the indispensable holistic understanding of the complex adaptive responses and tolerance determinants to relevant stresses, at a genome-wide scale. The knowledge thus achieved can be explored to guide the rational design of more robust microbial strains with improved performance for Industrial and Environmental Biotechnology or to overcome and control the deleterious activities of microorganisms in the Food Industry and in the Health sector. Moreover, using a microbial toxicogenomics approach, it can contribute to the understanding of mechanisms underlying the toxicity of and resistance to, a wide range of drugs and xenobiotics and other stresses, in more complex and less accessible eukaryotes, in particular in Plants and Humans. Throughout this communication, examples will be given on the research work carried out in the referred context by my research team, in the Biological Sciences Research Group (BSRG) of the iBB- Institute for Bioengineering and Biosciences at Instituto Superior Técnico (IST), Universidade de Lisboa (ULisboa).

RESUMO

Esta comunicação aborda um tópico de extrema importância em Biologia e Biotecnologia: as respostas e a tolerância de microrganismos a agressões ambientais, ao nível de todo o sistema microbiano. O seu foco é na abordagem contemporânea da Genómica Fisiológica Microbiana, que tem provado ser vital para se alcançar a indispensável compreensão holística das complexas respostas adaptativas e dos determinantes de tolerância a stresses relevantes, à escala do genoma. O conhecimento assim obtido pode ser explorado para guiar o desenho racional e construção de estirpes microbianas mais robustas e com melhor desempenho para aplicações em Biotecnologia Industrial e Ambiental ou para desenhar novas estratégias para controlar as suas atividades prejudiciais quer na Indústria Alimentar quer no

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setor da Saúde. Acresce que tem elevado potencial para contribuir para a compreensão dos mecanismos subjacentes à toxicidade e resistência a uma vasta gama de fármacos e xenobióticos e outros stresses, usando uma abordagem de Toxicogenómica Microbiana, em eucariontes mais complexos e menos acessíveis, em particular em Plantas e Humanos. Ao longo desta comunicação serão apresentados alguns exemplos do trabalho de investigação realizado, no referido contexto, pela minha equipa de investigação, no Grupo de Investigação em Ciências Biológicas (BSRG) do iBB - Instituto de Bioengenharia e Biociências do Instituto Superior Técnico (IST), Universidade de Lisboa (ULisboa).

MICROBIAL PHYSIOLOGICAL GENOMICS OF THE RESPONSE AND TOLERANCE TO STRESS: INTRODUCTION TO THE TOPIC

What is the interest of studying the response and tolerance to stress?

There are certainly strong scientific reasons for understanding the complexity of the cellular responses and tolerance to stress. Indeed, this is one of the great challenges in Biology because the survival and performance of living beings depend on their ability to sense environmental changes and to respond adequately through the remodeling of genomic expression.

However, there are also reasons from the economic, environmental and public health point of views for studying the response and tolerance to stress. In fact, the failure of therapeutic actions (use of antimicrobial and antitumor agents), conservation of foods (use of preservatives), protection of agricultural crops (use of pesticides) results from adaptive responses and tolerance to environmental aggressions of different nature (drugs, xenobiotics, toxic metabolites and other chemical or physical stresses) (Sá-Correia, 2019). Moreover, the improvement of the productivity of biotechnological processes and the efficacy of bioremediation processes in environmental recovery/cleaning strongly depend on the use and construction of efficient and robust microbial strains capable of coping with multiple stresses and other challenges.

The bibliographic references in the very broad field of R&D on microbial stress responses and tolerance, even when the contemporary strategies of Functional and Comparative Genomics are used, are immense. Therefore, and because there are obvious limitations to the indication of an extremely high number of references in a text of this nature that is largely an opinion article about the area, a large part of the introductions to the different topics do not include references. Even in the case of examples selected from the scientific contributions of my research group, review articles were cited instead of the corresponding numerous original articles. Also, only a very small number of selected articles among the many that, strictly speaking, should have been indicated, are listed among the references.

Why are the genome-based analyses so powerful?

The recent rapid development of DNA and RNA sequencing techniques and the continued reduction of their costs to very low values implicate that the systematic and large-scale sequencing of genomes is no more out of reach of many research laboratories. However, the comparative analysis of all the genomes available and the functional analysis of novel genomic sequences will certainly continue to be a challenge as well as a precious source of information. The proper exploitation of this information will not only allow the gain of new biological knowledge but also to guide and leverage innovation in biotechnology (dos Santos *et al*. 2012; dos Santos and Sá-Correia 2015). Indeed, the acceleration of genome sequencing has

stimulated the understanding of genomic expression remodeling in response to environmental challenges, the identification of mRNA and non-coding RNA profiles, protein-nucleic acid interactions at a global scale, and the discovery of the most hidden aspects of the regulation of gene and genomic expression. In other words, it has boosted functional, structural and comparative genomics, metagenomics, epigenomics and metabolomics analyses. The exploitation and integration of data coming from the use of all the *Omics* technologies (e.g. chemogenomics, transcriptomics, proteomics, in particular quantitative proteomics and phosphoproteomics, interactomics, metabolomics, lipidomics, comparative genomics and metagenomics) to understand microbial responses and tolerance to stresses require the development and use of a wide range of bioinformatics tools (Fig 1). Genome-based Biology is currently a reality and vital to boost biological research and applications and the understanding of the cellular behavior at the system level.

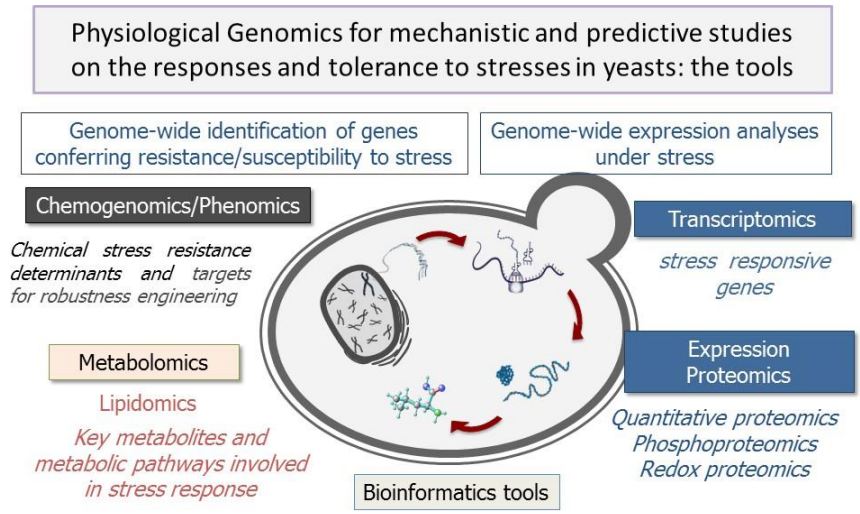


Figure 1
Omics analyses applied to Yeast to obtain mechanistic insights on the responses and tolerance to stresses, at a system's level.

Why studying the response and tolerance to stress in microorganisms is so important, even when they are not the true targets for specific stresses?

Microorganisms are essential to solve society's most pressing problems towards a sustainable bioeconomy. They are required to allow biotechnological developments for the substitution of fossil combustibles and other chemicals by biofuels and added-value chemicals produced by microorganisms from renewable resources and residues (the Petroleum Refinery *versus* the Biorefinery**)**. They also are biological agents for environmental recovery/cleaning through bioremediation and for the biotechnological production of foods, beverages and drugs. Moreover, it is estimated that a quarter of all deaths in the world result from infectious diseases caused by pathogenic microorganisms. Therefore, the understanding of the mechanisms of action and adaptive responses to specific stresses occurring either during the

corresponding bioprocesses or in the human host environment and their relevance to biotechnological productivity or to pathogenesis is essential to allow the development of more efficient bioprocesses or effective therapies.

Recent advances in Modern Biology, namely in the fields of Functional and Comparative Genomics and Systems and Synthetic Biology, would not have been possible without the use of model microorganisms. The most studied eukaryotic model is the yeast species *Saccharomyces cerevisiae* with a dual role also as a microbial cell factory of high relevance in Biotechnology, for the production of foods (e.g., bread), alcoholic beverages (e.g., wine and beer), bioethanol and other value-added chemicals, biopharmaceutical recombinant proteins. The budding yeast shares with human and plant cells many essential biochemical and physiological functions that were conserved during evolution.

Yeast has the full genome sequence available since 1996 having, since then, pioneered all post-genomic analyses (Goffeau *et al.* 1996; Goffeau 2000) (Fig 1, Fig 2). Coordinated by André Goffeau, a consortium of almost one hundred laboratories and private companies dispersed all over Europe took part in the yeast sequencing work sponsored by the European Commission. This effort was followed by another European Initiative to Uncover the Cellular Function of New Yeast Gene funded by the European Commission (the EUROFAN projects) (Fig 2). The success of this paradigmatic European research strategy, based on a distributed model of scientific collaboration, allowed my research group to join the EUROFAN projects. In this framework, a research programme, still active in my group, was started envisaging the functional analysis of novel yeast transporters of the major facilitator superfamily (MFS) (mediating active solute transport dependent on the transmembrane electrochemical potential) required for resistance to multiple drugs (MDR) (Sá-Correia and Tenreiro 2002a). Today, after more than two decades of post-genomic research in *S. cerevisiae*, a more comprehensive understanding of the molecular mechanisms underlying this species response and adaptation to a very wide range of stresses is a reality (Botstein and Fink 2011).

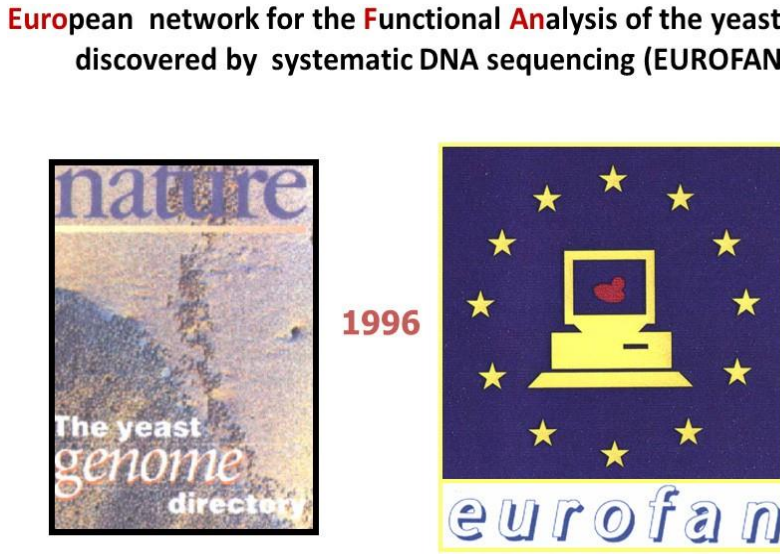


Figure 2
The EUROFAN projects funded by the European Commission followed the release of *S. cerevisiae* genome sequence, in 1996.

Despite the increased ability to conduct molecular and genomic research in more complex eukaryotes in the recent years, the yeast *S. cerevisiae* is still a powerful model eukaryote, both as a single cell experimental model organism and as a host cell for the expression and functional analysis of proteins from more complex and less accessible eukaryotes, including humans. As a proof of concept, a few examples on the translation of the knowledge obtained in the response and tolerance to herbicides in the yeast model to the plant model *Arabidopsis thaliana* will be reported later in this communication.

What is the power of Systems and Synthetic Biology strategies?

Microbial cells respond in a highly complex way to changes occurring in their environment and in their genomes. Despite all the progress made and the enormous wealth of data obtained during two decades of post-genomic research, a detailed understanding of all cellular functions remains unveiled. The challenge of trying to understand the complexity of biological systems is enormous, requiring a non-traditional view of molecular biology that bridges the genotype to the phenotype, given that the products of the various genes are not isolated functional units in a cell (Fig 3). Indeed, they work in molecular complexes, are sequentially organized in cascades and signaling pathways and in protein networks interacting physically and biochemically. The physical characterization of these networks, their regulation in space and time, and the response to different stimuli determine the function and dynamic characteristics of biological systems at the cellular level.

The new interdisciplinary area of Molecular Systems Biology aims to understand and predict the functioning of such complex biological systems considering all their molecular components and interactions (Fig 3). It is based on the integrative application of the principles and experimental strategies of molecular and cellular biology, functional genomics, chemogenomics, transcritomics, proteomics, metabolomics, mathematics, computational biology. Understanding and control the complexity of cellular behavior is essential for the resolution of complex biological questions and for the treatment of diseases and for solving the most pressing problems of our society in food production and conservation, energy production, and in health and agriculture.

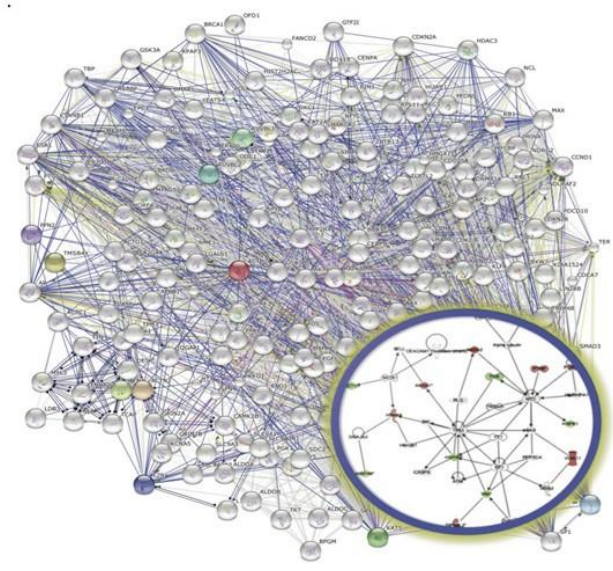


Figure 3
The products of single genes are not isolated functional units in a cell but work in protein networks interacting physically and biochemically. The figure was obtained using STRING (https://string-db.org/).

The contemporary use of genome-based approaches is also crucial for the exploration of Synthetic Biology strategies envisaging a more complete and integrated understanding of biological systems and the redesign and reconstruction of microbial strains capable of exhibiting functions more appropriate to specific biotechnological applications to achieve a sustainable bioeconomy or for understanding and explore the mechanisms of microbial pathogenicity control. Current advances in the Synthetic Biology field have resulted from the biological knowledge revealed by functional genomic strategies and from novel genomic manipulation techniques based on the CRISPR system that are allowing genome editing in an unprecedented way (Doudna and Charpentier 2014; Tian *et al*. 2017).

Microbial Physiological (Toxico) Genomics

Physiological Genomics exploits a wide variety of experimental and computational approaches, in particular the so-called Omics analyses and bioinformatics, to link genes to functions of complex biological pathways, as it is the case of those involved in the response and resistance to stress in microorganisms (Fig 1).

A number of recent studies demonstrate the power of Microbial Toxicogenomics approaches to directly assess the earliest stages of the toxicological response to cytotoxic insults and its potential for predictive toxicology and for guiding bioremediation strategies. In particular, Yeast Physiological Toxicogenomics (dos Santos *et al*. 2012; dos Santos and Sá-Correia 2015) provides a holistic assessment of the complex cellular adaptive responses to chemical toxicants in this eukaryotic model and cell factory. Such knowledge is instrumental for mechanistic insights to find targets for synthetic pathway engineering in yeasts (*S. cerevisiae* and non-conventional yeasts) for cell robustness manipulation for biotechnological applications or for designing novel strategies to control the deleterious activities of pathogenic or food spoiling yeasts in the presence of antifungal agents (clinical and agricultural fungicides and food preservatives). Since the majority of the chemical compounds in commercial use have not been comprehensively tested for human toxicity, Yeast Toxicogenomics is important to obtain such genome-wide view on the responses to chemical stresses and other environmental alterations relevant in Environmental Health, Pharmacology and Biotechnology (Fig 1). The use of the eukaryotic model *S. cerevisiae* has been instrumental to characterize new signalling pathways, understand and model gene regulatory networks under chemical stress, and identify molecular biomarkers of drug/toxicant exposure (Fig 1).

PHYSIOLOGICAL GENOMICS STRATEGIES TO UNDERSTAND MICROBIAL RESPONSE AND TOLERANCE TO STRESS: RECENT EXAMPLES FROM OUR RESEARCH

Response and tolerance to multiple stresses in yeasts: impact in Biotechnology, Food Industry, Agriculture and Environmental Health

The improvement of the capacity of industrially relevant yeast strains to tolerate toxic substrates or products combined with operating conditions that do not allow maximum stress tolerance, is an important challenge of modern Biotechnology (Fig 4) (dos Santos *et al*. 2012; dos Santos and Sá-Correia 2015; Godinho and Sá-Correia 2019). For example, the major challenge in the production of next-generation biofuels via yeast fermentation is to improve production yields and overcome the

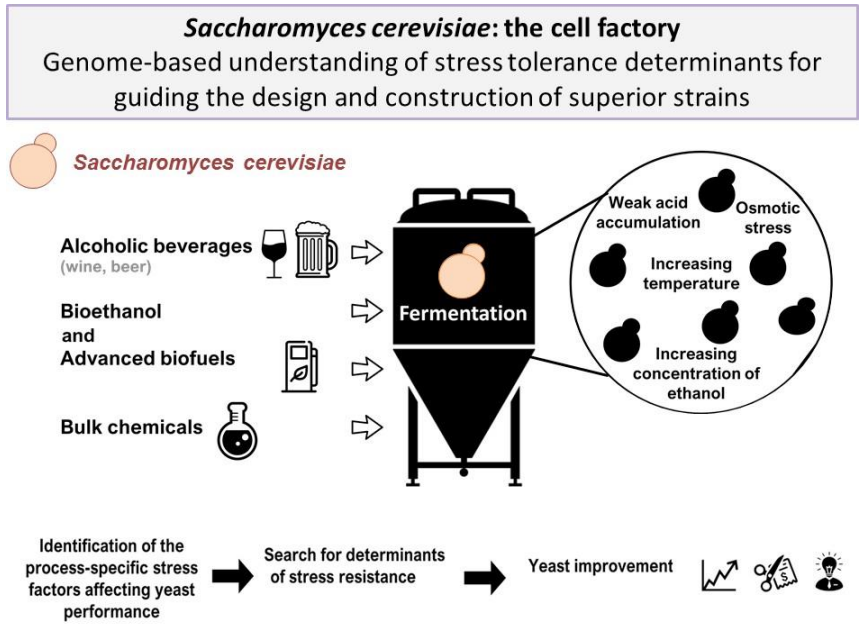


Figure 4
The improvement of the capacity of industrially relevant yeast strains to tolerate toxic substrates or products, combined with operating conditions that do not allow maximum stress tolerance, is an important challenge of modern Biotechnology. Prepared by Cláudia P. Godinho, BSRG – iBB.

technical and scientific challenges posed by lignocellulose-based processes, including fermentation inhibition by end-products and other compounds generated during hydrolytic treatment of raw materials (Fig 4) (Teixeira *et al*. 2009b; Mira *et al*. 2010c; Pereira *et al*. 2011; dos Santos *et al*. 2012; Remy *et al*. 2012; Pereira *et al*. 2014; dos Santos and Sá-Correia 2015). In this context, yeast toxicogenomic strategies have been fundamental to provide a more in-depth understanding of the mechanisms involved in yeast tolerance to relevant stresses and essential to identify their molecular targets and guide the design of more robust industrial strains. This knowledge is instrumental to guide synthetic pathway engineering for increased cell robustness manipulation either for the sustainable production of fuels and chemicals or for the control of growth and activity of food and beverage spoilage yeasts (Teixeira *et al*. 2011; Palma *et al*. 2015; Palma *et al*. 2018).

Chemogenomic, transcriptomic (quantitative- and phospho-) proteomic, lipidomic and genome sequence analyses, complemented by the use and development of the required bioinformatics tools and molecular and cell biology studies, have been explored in our laboratory to unveil genome-wide adaptive response programs and tolerance/susceptibility determinants to single and multiple relevant stresses in the cell factory *S. cerevisiae* (Teixeira *et al*. 2009b; Mira *et al*. 2010c; Pereira *et al*. 2011; Teixeira *et al*. 2011; Teixeira *et al*. 2012b; dos Santos and Sá-Correia 2015; Palma *et al*. 2018) and, more recently, in the highly weak acid tolerant food spoilage yeast *Zygosaccharomyces bailii* (Mira *et al*. 2014; Palma *et al*. 2015; Palma *et al*. 2017; Palma *et al*. 2018). These physiological genomics studies are identifying candidate molecular targets for genetic manipulations to endure/sensitize yeast cells against multiple stresses expected to occur during biotechnological and food industry processes to construct superior

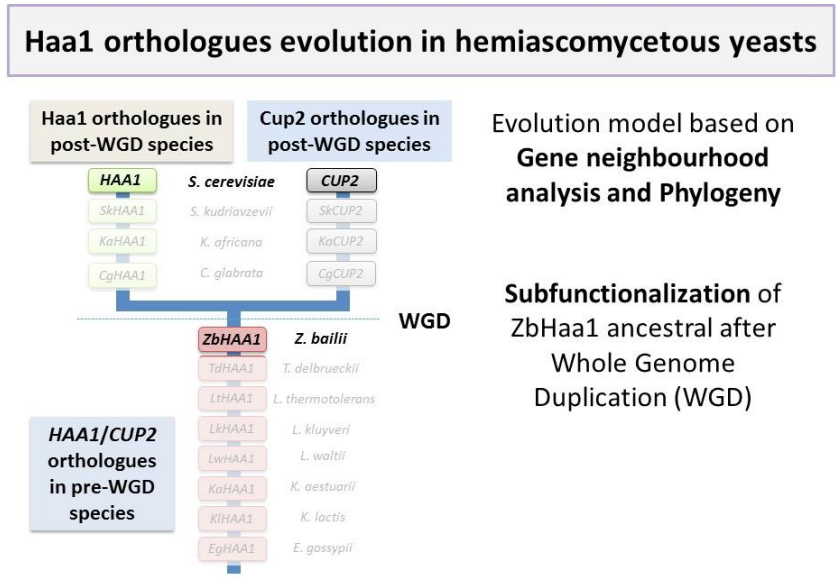
yeast strains (Teixeira *et al*. 2009a; Mira *et al*. 2010b; Teixeira *et al*. 2010; Pereira *et al*. 2011; Teixeira *et al*. 2012a; Pereira *et al*. 2014; Palma *et al*. 2015; Godinho *et al*. 2018).

The toxicological outcome of sudden or chronic exposure to environmental pollutants is scarcely understood at the molecular and cellular levels. Our Toxicogenomics studies on environmental pollutants involved agricultural agrochemicals, the fungicide mancozeb and the herbicide 2,4-D (Teixeira *et al*. 2005; Teixeira *et al*. 2006a; Teixeira *et al*. 2007; Santos *et al*. 2009; Dias *et al*. 2010; Cabrito *et al*. 2011). Mancozeb, a mixture of manganese- and zinc-ethylene-bis-dithiocarbamate (Mn:Zn, 9:1), is widely used against phytopathogenic fungi in several crops and vineyards. Although displaying low acute toxicity, the chronic exposure to this fungicide has recently been related to the development of environmentally-induced Parkinson's disease and certain forms of cancer. Our yeast toxicogenomic studies indicate that mancozeb acts as a thiol-reactive compound leading to massive protein oxidation and showed that 70% of the proteins differently expressed in response to mancozeb in the yeast model and 53% of the determinants of yeast resistance to this fungicide possess human orthologs (Santos *et al*. 2009; Dias *et al*. 2010; dos Santos and Sá-Correia 2015). Among them, are the targets of the major oxidative stress regulator in yeast, Yap1. Remarkably, the human orthologs of Yap1, Jun, and Jdp2, are activated during acute and chronic phases of several neurodegenerative diseases.

Although 2,4-D, an auxin-like synthetic herbicide, is one of the most successfully and widely used herbicides, its intensive use has led to the emergence of resistant weeds and might give rise to severe toxicological problems. Mechanistic insights into the global analysis of 2,4-D toxicity and the corresponding adaptive responses were obtained based on studies carried out using *S. cerevisiae* and *Arabidopsis thaliana* as model organisms (Teixeira *et al*. 2007). Studies also highlight the similarities of toxicological effects of these pesticides from yeast to higher eukaryotes, such as humans and plants. Hence, the use of the yeast model system is expected to continue to contribute to the understanding of the molecular mechanisms underlying pesticide toxicity in more complex and less easily accessible eukaryotes.

Transcription regulation of Gene and Genomic expression in Yeasts

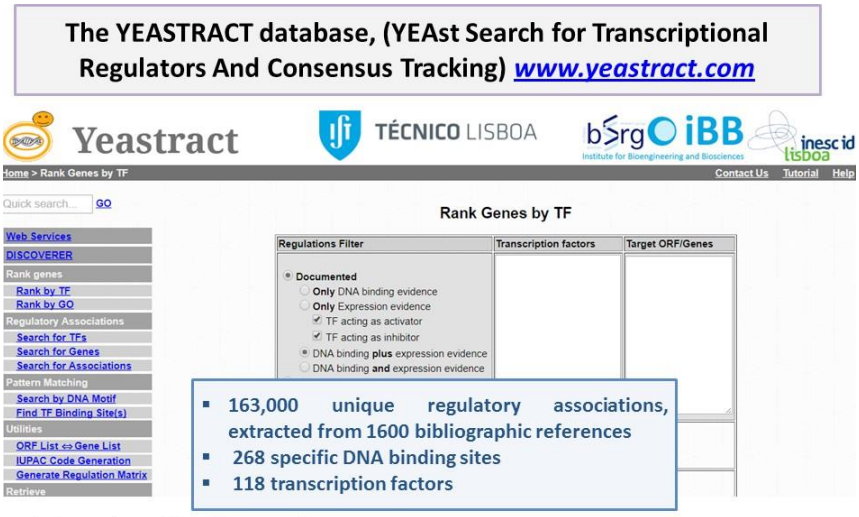
The rapid and adequate reprogramming of yeast genomic expression in response to environmental alterations, in particular to stresses, is essential for cell survival or metabolic efficiency. We have been examining and defining yeast regulons dependent on specific transcription factors based on transcriptomic analysis and identifying the corresponding DNA-binding sites and manipulating the corresponding regulons to increase stress tolerance. The most important example is the transcription factor Haa1 required for *S. cerevisiae* response to acetic acid stress, a highly important microbial inhibitory compound in Food Industry and Biotechnology (Fernandes *et al*. 2005; Mira *et al*. 2010a; Mira *et al*. 2010c; Mira *et al*. 2011a; Swinnen *et al*. 2017). In the non-conventional yeast species *Z. bailii* (Palma *et al*. 2018), remarkably tolerant to acetic acid, ZbHaa1, the functional homologue of *S. cerevisiae* Haa1 was found to be a bifunctional transcription factor able to modulate *Z. bailii* adaptive response to acetic acid and copper stresses, assuming the functions of *S. cerevisiae* paralogues Haa1 and Cup2 originated after the whole genome duplication (WGD) event (Fig 5) (Palma et al. 2017; Antunes *et al*. 2018; Palma and Sá-Correia 2019).



Palma MP, Dias PJ, Roque FC, Luzia L, Guerreiro JF and Sá-Correia I, ***BMC Genomics,*** 18: 75, 2017

Figure 5

The spoiling yeast *Zygosaccharomyces bailii* bifunctional transcription factor ZbHaa1, able to modulate the adaptive response to acetic acid and copper stresses, assumes the functions of *S. cerevisiae* paralogues Haa1 and Cup2 originated after the whole genome duplication (WGD) event.



Teixeira et al., ***Nucleic Acids Research*** 34: D446-D451, 2006
Monteiro et al., ***Nucleic Acids Research*** 36: D132-D136 2008
Abdulrehman et al., ***Nucleic Acids Research,*** 39: D136-D140, 2011
Teixeira et al. , ***Nucleic Acids Research,*** 42: D161-D166, 2014
Teixeira, Monteiro et al, ***Nucleic Acids Research,*** 46: D348–D353, 2018

Figure 6

The public YEASTRACT database (http://www.yeastract.com/). YEASTRACT provides a set of queries to search and retrieve important biological information from the gathered data and to predict transcription regulation networks in yeast from data emerging from gene-by-gene analysis or genome-wide approaches. YESTRACT was very recently extended to the other Yeasts of clinical and biotechnological interest in the YEASTRACT + portal (http://www.yeastract.com/) for cross-species comparative genomics of transcriptional regulation in Yeasts.

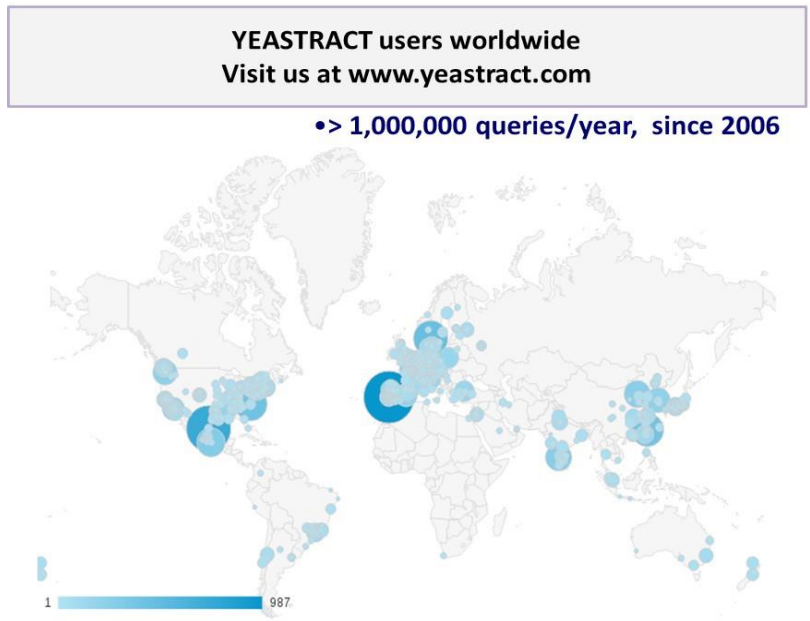


Figure 7
World-wide distribution of YEASTRACT users.

In collaboration with a research team affiliated to INESC-ID, we have developed and are regularly upgrading and updating the YEASTRACT (YEAst Search for Transcriptional Regulators And Consensus) database (www.yeastract.com) (Fig 6) (Teixeira *et al*. 2006b; Teixeira *et al*. 2014; Teixeira *et al*. 2017), an information system that has been providing free access to all published information on transcriptional regulation in the model eukaryote and cell factory *S. cerevisiae*, curated by experts in the field, for more than a decade. It is a key tool for the analysis and prediction of transcription regulatory associations at the gene and genomic levels in *S. cerevisiae,* very useful to analyze datasets coming from genome-wide expression experiments. Bioinformatics tools that enable the user to exploit the existing information to predict the transcription factors involved in the regulation of a gene or genome-wide transcriptional response and promoter analysis tools and interactive visualization tools for the representation of transcription factor regulatory networks are also provided. YEASTRACT has become an essential tool not only for yeast molecular biologists but also for systems biologists working worldwide to develop models of regulatory networks, as suggested by this World-wide distribution of YEASTRACT users (Fig 7).

Yeastract was very recently extended to the pathogenic yeasts *Candida albicans* and *C. glabrata* in the PathoYeastract (http://pathoyeastract.org/) (Monteiro *et al*. 2017) and later to the pathogenic species *C. parapsilosis* and *C. tropicalis* and the other non-convencional yeasts of biotechnological relevance: *Zygosaccharomyces builii*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* and *Kamagataella phaffii* (N. C. Yeastract; http://ncyeastract.org/). These extensions were conducted in the frame of the Portuguese distributed infrastructure for biological data BioData.pt, included in Fundação para a Ciência e a Tecnologia (FCT-funded) Infrastructure Road Map of 2013 (https://biodata.pt/). The resulting YEASTRACT+ platform for gene and genomic transcription regulation in yeasts is a service of the Portuguese Node of ESFRI-ELIXIR (European Distributed Infrastructure for Life Science Information).

Multidrug resistance transporters: biological function, regulation and evolution

Multidrug/Multixenobiotic resistance (MDR/MXR) is a widespread phenomenon with clinical, agricultural and biotechnological implications, where MDR/MXR transporters (of the major facilitator superfamily -MFS and the ATP-binding cassette Superfamily (ABC) play a key role in the acquisition of resistance (Balzi and Goffeau 1994; Sá-Correia *et al*. 2009). Although these proteins have been traditionally considered drug exporters, their physiological function and involvement in resistance to cytotoxic compounds are still open to debate (Godinho *et al*. 2018; Linton 2007; Sá-Correia *et al*. 2009; dos Santos *et al*. 2014). We have been contributing to the field since the release of *S. cerevisiae* genome sequence in 1996 by examining the biological function, regulation and evolution of poorly characterized MDR/MXR transporters (Sá-Correia and Tenreiro 2002b; Sá-Correia *et al*. 2009; dos Santos *et al*. 2014). In particular cases, the potential biotechnological application of these transporters to improve yeast robustness was also explored.

Heterologous expression of *S. cerevisiae* MDR/MXR transporters encoding genes in the plant model *Arabidopsis thaliana* and vice-versa was also explored to enlighten the biological role of these proteins in planta, in collaboration with the Plant Molecular Biology group of Instituto Gulbenkian de Ciência (Fig 8) (Cabrito *et al*. 2009; Remy *et al*. 2012; Remy *et al*. 2013; Remy *et al*. 2017). Particular attention was given to the response and resistance to agriculturally-relevant stresses (e.g. herbicides and other agrochemicals, high concentrations of toxic cations, phosphate limitation, drought) exploring the knowledge obtained in the yeast model and using yeastract as an expression host system to characterize the role of plant membrane MDR/MXR transporters (Remy *et al*. 2012; Remy *et al*. 2013; Remy *et al*. 2015; Remy *et al*. 2017). Given the conservation of transport mechanisms from *S. cerevisiae* to plants, our results validate the exploitation of the model yeast to uncover the function of plant MDR/MXR transporters an essential knowledge for the development of efficient strategies to improve crop productivity (Godinho and Sá-Correia 2019) (Fig 8).

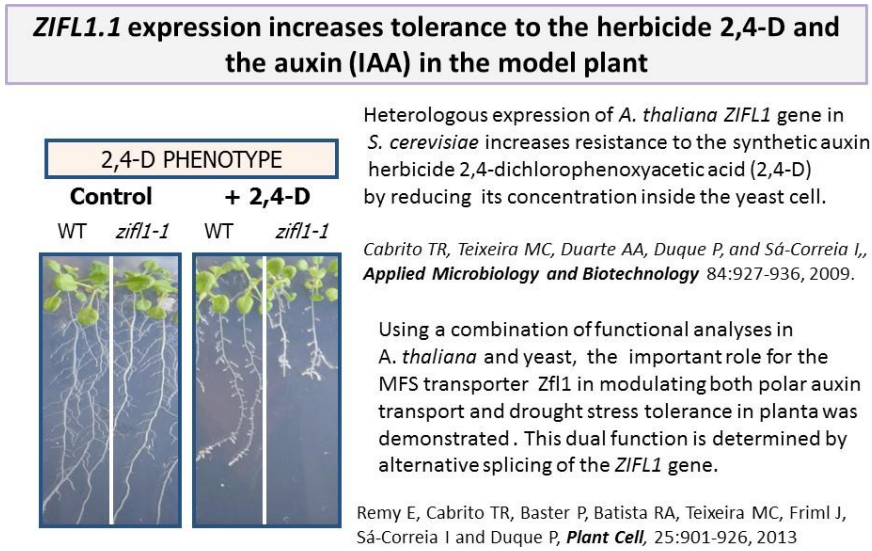


Figure 8
Phenotypic effects of the herbicide 2,4-D in *Arabidopsis thaliana* plant model, showing the in vivo protective role of the expression of the major facilitator superfamily (MFS) transporter Zfl1, demonstrated to play a dual role in auxin transport and drought stress tolerance.

Burkholderia cepacia complex bacteria in cystic fibrosis respiratory infections and as contaminants of pharmaceutical products

The *Burkholderia cepacia complex* (Bcc) comprises more than 20 closely related bacterial species that require a combination of molecular procedures for correct classification. These bacteria have a widespread environmental distribution, exhibit an extraordinary metabolic versatility, a complex genome with 3 chromosomes, an inherent resistance to multiple antibiotics and antiseptics and capacity to grow in nutritionally limited environments and for rapid mutation and adaptation. Due to this remarkable tolerance to multiple stresses, this group of bacteria adapt to the stressing cystic fibrosis (CF) lung environment being virtually impossible to eradicate and generally leading to a more rapid decline in lung function and, in some cases, to a fatal necrotizing pneumonia. Bcc bacteria are inherently resistant to multiple antibiotics severely limiting their eradication from the CF lung and antibiotic resistance of the early infecting strain of different Bcc species increases in late isolates (Leitão *et al*. 2008; Coutinho *et al*. 2011; Madeira *et al*. 2011; Mira *et al*. 2011b). The myriad of stresses encountered in the pathogens' hosts, may elicit a variety of adaptive protective responses with impact in innate antimicrobial susceptibility (Fig 9).

Also, several Bcc outbreaks were linked to their resistance to antiseptics, in particular benzalkonium chloride, commonly used in pharmaceutical formulations. Indeed, Bcc bacteria are feared as contaminant of pharmaceutical and personal care products and the frequent microbial contaminant in non-sterile products recalls (Cunha *et al*. 2007; Coutinho *et al*. 2015). Contaminations with Bcc have caused nosocomial outbreaks in healthcare facilities and pose a health threat for susceptible individuals, in particular cystic fibrosis (CF) patients (Cunha *et al*. 2007; Coutinho *et al*. 2015).

Our research group has a long-term experience in the elucidation of relevant aspects of Bcc-mediated respiratory infections in CF patients, resulting from a two-decade long collaboration with the major

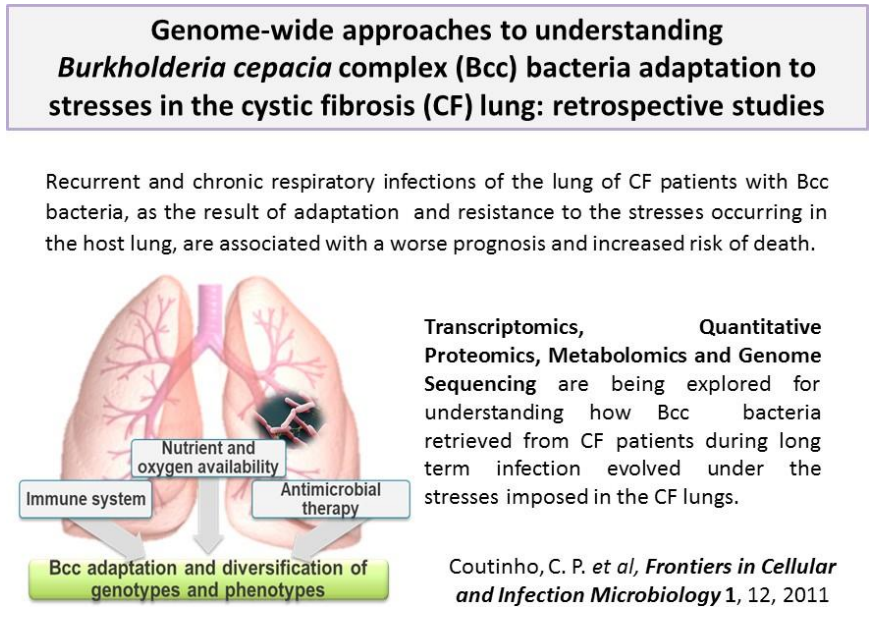


Figure 9
Pathogenomics of *Burkholderia cepacia complex* bacteria in lung infections in cystic fibrosis patients.

Portuguese CF Centre at Hospital Sta Maria, in Lisbon, and in the availability of around one thousand clinical isolates recovered from Bcc-infected patients during chronic infection (Cunha *et al*. 2003; Cunha *et al*. 2007; Correia *et al*. 2008; Leitão *et al*. 2008; Coutinho *et al*. 2011; Coutinho *et al*. 2015). Clones from the poorly represented *B. cepacia* and *B. contaminans* species among the CF populations characterized worldwide, were epidemiologically related with clones detected, in 2003 and 2006 in non-sterile saline solutions for nasal application (Cunha *et al*. 2007; Coutinho *et al*. 2015). For the last decade our studies contributed to increase our understanding on the mechanisms underlying Bcc bacteria capacity for causing persistent and devastating respiratory infections in CF patients. Retrospective studies focused on long term adaptive evolution of different species in the CF lung using functional comparative and genomics have been performed (Fig. 9). An integrated molecular systems microbiology strategy, including genome-wide expression analysis (by transcriptomics and quantitative proteomics profiling), metabolomics, high-throughput sequencing, phenotypic and biochemical characterization and molecular and physiological studies on selected isolates has been explored (Madeira *et al*. 2011; Mira *et al*. 2011b; Madeira *et al*. 2013; Moreira *et al*. 2016; Hassan *et al*. 2017; Moreira *et al*. 2017) . More recently, our attention is also focusing on the mechanism behind the success of Bcc bacteria, especially the rarer and less studied species, as health products' contaminants.

FINAL REMARKS

The new Biology is based on the synergy of interdisciplinary research at the intersection of biological sciences with engineering, computer science, mathematics, physics and chemistry, among other areas of knowledge. Such revolution in the way how scientific knowledge is currently obtained and in how innovation in the field of Life Sciences develops, also presupposes a change in the way modern biology is taught and the scientific knowledge is obtained. Concerning the broad field of the responses and tolerance to stresses, the exploitation of cutting-edge post-genomic approaches is vital to ensure research competiveness and leverage innovation in Industrial, Environmental and Health Biology and Biotechnology areas.

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Um pedido de desculpa por todas as referências bibliográficas que ficaram por citar.

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