

Meeting report

Human fungal pathogens accelerate into the genomics era

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A report on the Euroconference on Fungal Virulence Factors and Disease: 'Human Fungal Pathogens', Seefeld, Austria, 8-13 September 2001.

The common human pathogen *Candida albicans* was the main focus of the recent Euresco conference on 'Human Fungal Pathogens'. This fungus encounters a number of host niches during the course of infection, and its ability to adapt to the changing environment is a key feature of virulence. Whole-genome analysis featured in the majority of talks by invited speakers, who discussed the various ways by which *C. albicans* adapts to changes in its environment.

Sensing a change

The ability to undergo a switch from a yeast to a hyphal growth form is important for the pathogenicity of *C. albicans* (Figure 1). At least two conserved signaling cascades that are involved in this switch have been identified: the pathways mediated by the mitogen-activated protein kinase (MAP) kinase and by cAMP-dependent protein kinase A (PKA), respectively. These signaling pathways often converge on common genes that are involved in differentiation. In *C. albicans*, the MAP kinase and PKA pathways provide signals for filamentation via regulation of at least two known transcription factors - Cph1p and Efg1p. Jesús Pla (University Complutense of Madrid, Spain) presented epistasis studies that highlighted the potential for cross-talk between the pathway mediated by the MAP kinase HOG (high-osmolarity glycerol), which signals the response to hyperosmotic stress in yeast, and other stress-response pathways, specifically the oxidative-stress cascade, as well as possibly different morphogenetic pathways. Joachim Ernst (Heinrich-Heine-University, Düsseldorf, Germany) discussed the distinct roles played by the PKA isoforms Tpk1p and Tpk2p during the hyphal switch in *C. albicans*. Mutants deficient in *tpk1* showed defective hyphal development on solid inducing

media but only a slight defect in liquid inducing media, whereas mutation of *tpk2* had the opposite effect. Domain-swapping experiments between Tpk1p and Tpk2p revealed that the carboxy-terminal catalytic portions are responsible for filamentation in response to different media. In contrast, the amino-terminal domain of Tpk2p is responsible for agar invasion (another phenotype associated with the morphological change to hyphal growth).

A variety of environmental stimuli promote hyphal development in *C. albicans*, and Carol Kumamoto (Tufts University, Boston, USA) described the stimulus provided by interaction with the surrounding matrix. Embedding cells in a matrix, such as agar, agarose or gelatine, is sufficient to promote the development of true hyphae. This is a purely physical stimulus, because effects of oxygen limitation or

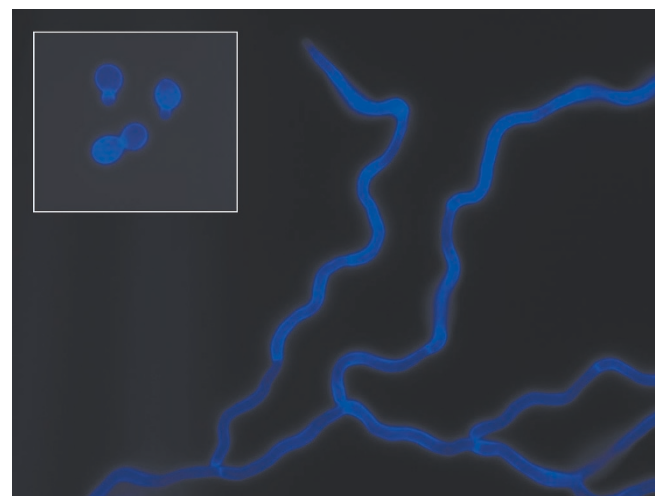


Figure 1

Two growth forms of *C. albicans*. The hyphal growth form is shown in the main picture and yeast forms are shown in the inset image. The cells were stained with calcofluor and photographs were taken at 630x magnification.

nutrient limitation were eliminated experimentally. The transcription factor Czf1p has been identified as being important for filamentation during growth within a matrix: ectopic expression of Czf1p causes precocious hyphal development and *czf1* deletion delays filamentation. Czf1p interaction with the transcriptional co-activator Wcn1p may be required for activation of filamentation genes. Epistasis and microarray studies have indicated that Czf1p may act by influencing the activity of the Efg1p transcription factor, and Czf1p and Cph1p may be partially redundant.

A new transcription factor (Cph2p) was introduced by Haoping Liu (University of California at Irvine, USA). Cph2p is involved in the induction of hyphae-specific genes but operates independent from the known Cph1p-mediated MAP kinase or Efg1p-mediated cAMP/PKA-dependent pathways. Cph2p function is mediated, at least in part, through the transcription factor Tec1p, as ectopic expression of Tec1p suppresses the defects in hyphal development seen in the *cph2* mutant. DNA-array results indicate that distinct filamentation signaling pathways converge to regulate a common set of genes. The use of transcript profiling described by Alistair Brown (University of Aberdeen, UK) has identified new potential targets of the morphogenetic signaling pathways. Brown showed that there is some coordinated expression of morphogenetic and other virulence factors, suggesting that *C. albicans* utilizes convergent regulation of vital virulence factors to ensure survival and pathogenicity in various host environments.

Within the host

Although we can dissect the morphogenetic signaling pathways *in vitro*, the important question of what happens during infection still remains. Joachim Morschhäuser (University of Würzburg, Germany) described his use of *in vivo* expression technology (IVET) in mouse. In this approach the activation of a target gene in an infected host results in the expression of the site-specific FLP recombinase, which in turn catalyzes the excision of a resistance marker, in this case mycophenolic acid (MPA). Expression and hence excision of the MPA marker results in MPA-sensitive cells after re-isolation from the infected host. *C. albicans* has large gene families encoding proteins with virulence-related functions, suggesting the evolution of family members that may be important at different stages of infection. Morschhäuser showed that different types and stages of infection correlate with differential expression of members of the secreted aspartic proteinase (SAP) gene family. Positive results in this type of study require only transient expression, and this may explain why the results differ from previous *in vitro* experiments.

Transcriptional changes that result from *C. albicans* infecting macrophages was the topic discussed by Michael Lorenz (Whitehead Institute for Biomedical Research, Cambridge,

USA). Virulent *C. albicans* yeast cells undergo a morphological switch to the hyphal growth form after phagocytosis by macrophages. The hyphae are able to penetrate out of the phagolysosome, a macrophage compartment in which phagocytosed particles are usually degraded, and hence the fungus can evade this stage of the host defense. Cultured murine macrophages are also able to phagocytose *Saccharomyces cerevisiae*. To initiate studies, therefore, *S. cerevisiae* was used as a model system to examine early-stage transcriptional changes induced by phagocytosis. Whole-genome microarray analysis revealed that the primary response was the activation of the glyoxylate cycle. The genes encoding the two central enzymes involved in this pathway, *ICL1*, encoding isocitrate lyase, and *MLS1*, malate synthase, were found also to be induced in *C. albicans* upon phagocytosis. *C. albicans icl1* mutants are unable to use two-carbon compounds as the sole carbon source but are not otherwise stress-sensitive *in vitro* - but *icl1* mutants show a significant virulence defect *in vivo*. Enzymes from the glyoxylate cycle, which does not exist in mammals, have also been identified as virulence factors in *Mycobacterium tuberculosis* and hence are ideal candidates for antimicrobial drug development.

Genome flexibility

As previously suggested by Elena Rustchenko and colleagues (University of Rochester Medical School, USA), *C. albicans* has a unique mechanism whereby changes in chromosome copy number result in changes in expression levels of positive and negative regulators, and thus allow adaptation to stressful environmental conditions. Initial studies showed that in independent mutants for utilization of the alternative carbon source L-sorbose occurred concomitantly with a specific loss of either one of the two homologs of chromosome 5; usually, *C. albicans* has seven chromosomes. Rustchenko extended the study to show that specific chromosome alterations, such as duplications and precise truncations, are also seen when other nutritional pressures are applied, such as D-arabinose utilization. This phenomenon can also be seen when pressures arise from the presence of antibiotics such as fluconazole, where short- and long-term exposure results in distinct and specific chromosome alterations. Of particular interest to the majority of attendees was the revelation that 5-fluoroorotic acid (5FOA) also causes chromosome alterations at a relatively high frequency. This is of interest to people using 'URA blasting', a gene-disruption technique in which the recombination between short repeats flanking the *URA3* marker gene results in *ura3⁻* strains, which can be selected for by resistance to 5FOA. Because of the complicated diploid nature of *C. albicans*, URA blasting is used extensively as it allows recycling of the *URA3* marker. The frequency of 5FOA resistance due to correct recombination within the URA-blaster cassette is only one order of magnitude higher than that caused by chromosome loss.

Continuing the discussion of how genome alterations can influence the virulence of *C. albicans*, William Fonzi (Georgetown University, Washington, USA) showed that the defective filamentation phenotype originally identified in the *hwp1* mutant, which is deficient in the hyphal cell-wall protein HWP1, was in fact due to deficiencies in the expression of the transformation marker *URA3*. Filamentous sectors emanating from colonies of the null *hwp1* mutant were shown to have tandem duplications of *URA3* at the *HWP1* locus. By placing the *URA3* gene in different positions within the genome, it was shown that the filamentation defects were a result of the marker-gene location subtly influencing the expression of *URA3*, rather than of the *hwp1* mutation itself. Fonzi's talk sparked a general discussion in which further examples of position effects were described. David Soll (University of Iowa, Iowa City, USA) described how specific regulation of several reporter-gene constructs was lost when they were placed at ectopic positions in the genome. Thus, subtle differences in expression levels due to chromatin context may be important in determining virulence. These examples of how chromosome flexibility and positional context can influence fungal virulence highlighted the need to be extremely thorough in checking the karyotype, genotype and expression patterns of strains.

Mating and recombination in *C. albicans*

Until relatively recently, *C. albicans* was considered to be an asexual organism. Several groups have now identified *C. albicans* homologs of many of the genes required for mating in *S. cerevisiae*, however, including the mating-type-like loci *MTLa* and *MTL α* . Pete Magee (University of Minnesota, St Paul, USA) described how the generation of tetraploid strains can be forced *in vitro* by mating complementary auxotrophic strains that are homozygous at the mating-type-like loci. The *MTLa* and *MTL α* strains were as virulent as the parental strain, but the tetraploid strain resulting from *in vitro* mating was markedly less virulent than either the homozygous or heterozygous diploids. This raises the question of whether *C. albicans* can mate in the wild. Of 120 clinical isolates examined, four were homozygous or hemizygous for *MTL α* and six homozygous or hemizygous for *MTLa*. Co-inoculation into a mouse model of *MTLa* and *MTL α* differentially marked with auxotrophic mutations resulted in the recovery of prototrophic diploid reisolates. Magee suggested that instead of going through a haploid intermediate, *C. albicans* may go through a tetraploid intermediate, and chromosome loss may be the mechanism for reducing chromosome number.

Variation in the size of the major repeat sequences (MRSs) present in nearly all *C. albicans* chromosomes allows identification of individual chromosome homologs. Chromosomes carrying larger MRSs are lost at higher frequency. Genetic recombination also occurs, as linked genes from different

parents can appear in the homozygous form in the same reisolates. Gabriele Schönián and colleagues (Humboldt University, Berlin, Germany) performed population genetic studies of geographically different populations by identifying polymorphic loci within PCR fragments. The studies suggested that, although the populations studied showed primarily a clonal mode of reproduction, there was evidence for recombination. The mechanisms underlying recombination and chromosome reduction (either chromosome loss or meiosis) are as yet unknown, but the possibility of mating and recombination in *C. albicans* has great implications for studies *in vivo*, as well as providing an invaluable tool for studying this organism *in vitro*.

As *C. albicans* research explodes into the era of whole-genome technology, it is now up to the *Candida* community to sort through the biology that is embedded in the reams of information currently being produced. Participants at the 'Human Fungal Pathogens' meeting were given just a taste of what is to come.

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