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PERSPECTIVES

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Candida albicans molecular biology reaches its maturity

Introduction Opportunistic fungal infections have become a major threat for humans. The development of anti-inflammatory and immunosupressive chemotherapies to avoid graft rejection or cancer progression, the introduction of more aggressive medical procedures, including prostetic devices, the treatment of patients with broad-spectrum antibiotics and, more recently, the emergence of AIDS have resulted in a growing number of individuals with impaired host defenses. These immunocompromised patients are easy targets for invasive fungal infections which have become major causes of morbidity and mortality. Additionally, the use of a rather limited number of antifungal drugs has resulted in the appearance of antifungal drug resistance. Consequently, an intensive search for new antifungal agents has occurred. Of the several potentially invasive fungi, *Candida* spp. and *Aspergillus* spp., are responsible for many life-threatening infections, especially, *C. albicans* and *A. fumigatus.*

C. albicans is the most prevalent fungal pathogen of humans. As a commensal of the healthy individual, this fungus lives saprophytically in several niches of the human body. Alterations in the balance between the commensal and the host, as occurs in the immunocompromised patient, may trigger infection of the mucosal epithelia followed by dissemination via the bloodstream and colonization of internal organs. While *C. albicans* is the focus of intense research, knowledge of its biology has been hindered by the fact that it is an obligate diploid lacking a sexual cycle. Infection of mucosal epithelium of human tissues by *C. albicans* as well as disease progression seems to require a reversible conversion from a yeast form to a hyphal morphology. Hence, a large amount of molecular studies on *C. albicans* have focused on genes related to filamentation, which are also expected to be linked to virulence.

In USA, candidiasis is the third most common blood-borne infection, its incidence being especially high in neonates and Afroamericans. About 70% of adult women undergo at least one episode of vulvovaginal candidiasis during their lifespan. Oropharyngeal candidiasis occurs as an opportunistic infection in AIDS patients, and it has been estimated that 85–90% of these patients develop oral, esophageal candidiasis and/or vulvovaginitis. The current therapy is problematic. Candidiasis occurs as superficial (skin and mucosal membranes) or invasive infection. Superficial candidiasis occurs mainly in AIDS patients whereas the latter is common in cancer patients or individuals that have transplants following immunosuppression. The latter conditions are responsible for the high levels of mortality and morbidity. One of the reasons is that the diagnosis of the infection may be difficult since the symptoms (fever) are not specific. In Spain, the incidence of candidiasis by *C. albicans* in hospital patients is also increasing (from 3.1% of the isolates in 1990 to 4.07% in 1997). In addition, a significant rise in the number of isolates of *Candida* species different from *C. albicans* has been recorded in the same period (from 1.6% to 2.13%). Again, superficial infections are common in AIDS patients, who develop esophageal candidiasis with increasing incidence (from 14.34% in 1997 to 18.47% in 1999) according to data from Comunidad Autónoma de Madrid (Autonomous Government of Madrid).

Aspergillus fumigatus is also a familiar oportunistic pathogen able to cause life-threatening infections in the immunocompromised patient. *A. fumigatus* infections are not as common as *Candida* infections, since only 5 out of 100.000 healthy people develop aspergillosis, although this infection reaches a high incidence (about 10%) in bone-marrow transplants. Nevertheless, the outcome of this infection is usually serious since diagnosis is not made until death. As with *C. albicans,* the number of antifungals available for treatment is limited and, additionally, the organism is intrinsically resistant to fluconazole, a drug often used to treat candidiasis patients.

Factors suggested as contributory to the virulence of *A. fumigatus* include toxins, proteases and pigment production. It has also been possible to determine the virulence factors of this organism through molecular approaches. However, new antifungal agents are still sought.

A Symposium on the Molecular Biology and Pathogenesis of *Candida albicans* and *Aspergillus*, sponsored by Fundación Areces, was held in Badajoz, Spain, September 20–23, 2000. The central aim of this symposium was to gather national and foreign scientists to present and discuss the current data in their particular research fields. Due to the limitation in time and funds only a few topics relevant to the biology and pathogenicity of those fungi could be dealt with. These topics included: cell wall structure and biogenesis because of its role in pathogenesis and as potential targets of antifungal agents; extracellular sensors and signal transduction as a way to transmit environmental signals that change the behaviour of these opportunistic pathogens; dimorphism, because of its relevance to virulence and the intriguing role of the sexual locus in the asexual *Candida albicans*. Although several talks dealt with the behaviour, antigenicity, pathogenicity and the genome sequencing project of *A. fumigatus*, for space reasons, we will only refer here to new findings on *C. albicans*.

Cell-wall proteins The session was opened by Rafael Sentandreu (University of Valencia). He emphasized the discovery of new cell wall proteins which are thought to cooperate to the construction of the cell wall. A new *C. albicans* mutant, whose cell wall is devoid of antigen 3H8, a major epitope found in wild type cells regardless the morphology, yeast or mycelium, was discussed. The mutant was found in animal tissues but the adhesion ability of the cells was significantly reduced as compared with the wild-type, parental strain.

Mannoproteins are clearly involved in adhesion of *Candida* cells to oral mucosa or dental prosthesis, as shown by José Pontón, from the University of the Basque Country, Bilbao, Spain. A study which used two different experimental models showed that adhesion of *C. albicans* correlates with germination of yeast cells, since conditions which promote filamentation increase adhesion and those inhibiting germination depress it. Mutants unable to germinate show very low levels of adhesion to plastic materials (polystyrene) or to the cell line Hep-2c. Adhesion of *C. albicans* to oral surfaces may be modulated by different salivary components, especially secretory IgA (sIgA), the most important immunoglobulin present in the oral cavity. Saliva does not affect germination, but enhances the adhesion of *C. albicans* blastospores and decreases the adhesion of fully germinated cells. This inhibition is mediated by sIgA.

Monoclonal antibodies affect adhesion in different ways. Some of them either have no effect or enhance adhesion to polystyrene whereas others inhibit (like sIgA, but using a different mechanism) adherence of *C. albicans* to oral surfaces. Thus, monoclonal antibodies B9E and N3B inhibit the germination of *C. albicans*, and monoclonal antibody C7 kills fungal cells whereas sIgA blocks the adhesins present on the germ tube cell-wall surface. Although these monoclonal antibodies have not yet been tested in an in vivo model of mucosal candidiasis, monoclonal antibodies B9E and C7 are effective in prolonging the survival of mice injected intravenously with a lethal dose of *C. albicans*.

Response of *Candida albicans* to the external environment An important topic of the symposium was the analysis of the response of *C. albicans* to the external environment, a behaviour shared by all living organisms, especially when external changes are critical to growth or survival. The case of *C. albicans* is illustrative, since this pathogen copes successfully with a number of environmental variables as evidenced by its ability to occupy diverse niches in its human host. W. Fonzi, from Georgetown University, USA, described the role of the pH-response in the biology of *C. albicans*. The importance of this response is illustrated by the niche-conditional virulence phenotypes of mutants lacking certain pH-regulated genes or mutants defective in the regulation of pH-conditional gene expression. For instance, a *phr1*-Δ mutant, which lacks the alkaline-expressed gene *PHR1*, is avirulent in systemic infections where the host conditions are slightly alkaline, but fully infectious in the acidic rat vaginal environment. Several cellular processes can be affected by the external pH. Some of them correspond to basic cell functions such as the assembly of cell wall glucans via the glycosidases Phr1 and Phr2. Others, as *PRA1*, may be more directly involved in pathogenesis. Pra1p, which is expressed under alkaline conditions, is related to a prominent antigen of *Aspergillus* spp. which has been implicated in fibrinogen binding. On the other hand, some of the secreted enzymes which are thought to be involved in virulence such as SAP4, SAP5 and SAP6 (Secreted Aspartyl Proteases) are only synthesized at neutral pH. Finally, developmental transition from yeast to hyphae is also pH-dependent.

Regulation of expression of these pH-responsive genes is conserved in fungi. All of these genes are under the control of a zinc-finger containing transcription factor encoded by *RIM101*/*PRR2*. This is the ortholog of *pac*C of *A. nidulans*, *YlRIM101* of *Yarrowia lipolytica*, and *RIM101* of *Saccharomyces cerevisiae*, which regulates the pH-response of these species in a similar manner. PacC has been extensively studied by Peñalva's group at the Center for Biological Research of the Spanish Council for Scientific Research (CIB-CSIC), in Madrid. It is synthesized in an inactive form, which is activated by proteolytic removal of the C-terminus. Proteolytic activation occurs at alkaline pH and requires six "*pal*" genes. Genetic evidence supports the concept that the same kind of activation occurs in *C. albicans*, where the activated CaRim1p activates transcription of alkaline expressed genes and represses expression of acid expressed genes.

Signal transduction pathways R. Calderone, also from Georgetown University, reported recent studies on a two component signal transduction pathway in *C. albicans*. These kinds of systems are well known in prokaryotes, where they transmit signals through a histidine-kinase sensor and response-regulator proteins. The activated response regulator acts as a transcriptional regulator for genes encoding virulence factors or growth regulators. The recently identified *C. albicans* pathway is similar to the *SLN-YPD-SSK1* two-component system from *S. cerevisiae*. However, whereas *CaYPD1* and *CaSLN1* complemented the corresponding mutants in *S. cerevisiae*, *CaSSK1* did not. A second difference is that whereas the two-component signal transduction pathway is primarily responsible for the adaptation of *S. cerevisiae* cells to high osmolarity, *C. albicans* null mutants in *sln1* or *ssk1* are not osmosensitive, but have defects in morphogenesis instead. The presence of other putative genes encoding for histidine kinases, such as *CHK1* and *NIK/COS1*, complicates the situation, but again strains deleted in each of these genes are not osmosensitive but are defective in filamentation. Interestingly, null mutants in the genes encoding histidine kinases are either avirulent or attenuated in their virulence in a murine-disseminated model of candidiasis. A new hint on the requirement of different genes to colonize different environments was provided by the finding that the *chk1/chk1* mutant infected the vaginal mucosa although it was readily killed by white cells which are thought to be of importance in resistance to invasive disease but not vaginitis. Interestingly, *ssk1/ssk1* strains are less adherent to both HET1-A human esophageal cells and reconstituted human esophageous tissue, which indicates that they may be deffective in hyphae cell surface component(s). The fact that null mutants in *SSK1* and *CHK1* flocculate extensively supports this assumption. Further studies will define the pathway(s) as well as its main input and output.

Jesús Pla, from the Complutense University of Madrid, discussed the components of the high-osmolarity glycerol pathway (*HOG1*). *CaHOG1* not only complements *S. cerevisiae hog1* mutants, but its deletion causes the osmosensitive phenotype. These mutants are more resistant to antifungals that interfere with chitin assembly, which relates *HOG1* to the cell wall biogenesis. Mutants *hog* are more filamentous under a variety of conditions known to induce hyphae (pH, temperature, serum) but they are less virulent than a wild type parental strain, which indicates that filamentation is not absolutely linked to virulence. Epistatic studies with known transcription factors such as *RBF1*, *CPH1* and *TUP1* indicate that the overfilamentation phenotype depends on the presence of *CPH1*, but not *HST7*. Surprisingly, these investigators found, in *hog1* mutants, an enhanced susceptibility to oxidative stress induced by hydrogen peroxide or superoxide anions. The relationship of Hog1p and Cap1p, the homologue of the *S. cerevisiae* transcription factor Yap1p, which mediates response to oxidative stress, is currently being analyzed by this group.

Dimorphic transition Dimorphic transition was another topic of the symposium. Joachim F. Ernst, from Heinrich-Heine-University Düsseldorf, Germany, presented new material related to the *EFG1* pathway. In *C. albicans*, pseudohyphae are induced by starvation whereas hyphal development is induced by other signals. The bHLH transcription factor Efg1p regulates a number of morphogenetic pathways. Thus, null mutants in *EFG1* are unable to filament in most media and are defective in the formation of chlamydospores; in addition, low *EFG1* expression correlates with an elongated, opaque-like phenotype, whereas forced *EFG1* expression leads to the yeast-like white phenotype in strain WO-1. This *EFG1* pathway acts independently and is more determinant to morphogenesis than the MAP-kinase pathway represented by *HST7* and *CPH1*. According to recent studies, *EFG1* is a component of the cAMP-dependent signalling pathway. Starvation or other positive morphogenetic inducers, such as serum or $GlcNH_2$, are probably detected by a O-glycosylated sensor, since *pmt* mutants (*pmt1*, *pmt4* and *pmt6*) are defective in morphogenesis. The signal is probably conveyed through Ras1 to the adenylate cyclase system of the cell, since a *RAS* gene is required for morphogenesis. In fungi, the effector of the cAMP-dependent signalling pathway is the protein kinase A.

The *C. albicans* genome project has revealed the presence of two potential *PKA* genes, homologues to *TPK1* and *TPK2* from *S. cerevisiae*. Both *C. albicans* genes are involved in hyphal formation since each deletant showed defects in morphogenesis. By contrast, overexpression of either gene led to derepression in myceliation. Epistatic studies, such as overexpression of *EFG1* in *tpk1* or *tpk2* mutants or viceversa, are consistent with Efg1p being a downstream target of Tpk1 and/or Tpk2. Consistently with the existence of a phosphorylation cascade, site-directed mutagenesis has identified a putative phosphorylation site in Efg1p, since the mutant T206A blocked morphogenesis, whereas a T206E-mutation was hyperactive. Unfortunately, the direct target of Efg1p remains unknown, although the current data indicate that Efg1p is required for the expression of several hypha-specific genes. Interestingly, the *EFG1* promoter carries specific cis-acting elements that mediate Efg1p-dependent regulation. The basis of this autoregulation is complicated by the presence of two *EFG1* transcripts, a major one with an extensive 1.16 kb untranslated region and a minor counterpart with almost no 5´ untranslated codons. Deletion analysis have revealed that these transcripts respond to different cis-elements, although both are repressed by high Efg1p levels. Studies with the minor transcript have indicated that a E-box-like sequence present in the upstream regulatory region is dispensable for autoregulation, as is the case of *myc* autoregulation.

Al Brown, from the Aberdeen Fungal Group, Scotland, UK, presented interesting studies on the analysis of hypha specific promoters in *C. albicans*. A number of genes have been identified, including *ALS3*, *ALS8*, *ECE1*, *HYR1* and *HWP1*, whose expression parallels hyphal development induced under

various conditions in vitro. However, null mutants in each of these genes still undergo dimorphic transition, indicating that none of these genes are required for hyphal growth. Analysis of the *ALS8* promoter has identified different regions which mediate responses to different environmental stimuli. *ALS8* expression is absolutely dependent upon Efg1p and, in agreement with this, an E-box which appears to bind Efg1p is present in the *ALS8* promoter. However, this E-box is not sufficient for *ALS8* gene induction. A different region, which contains GCRE and YRE-like elements, is required for transcriptional activation in response to serum, and an alternative promoter region is required for *ALS8* induction in response to pH. In summary, different regions of the *ALS8* promoter mediate activation in response to different environmental stimuli, but Efg1 is required under all the conditions tested. On the other hand, *ALS8* (as well as *HWP1*, *ECE1*, and *HYR1*) is derepressed in *tup1/tup1* mutants (which grow as hyphae or pseudohyphae) even in the absence of hypha-inducing stimuli, an indication that this gene is under the control of the general repressor Tup1p.

A similar phenotype, i.e. constitutive hyphal morphology and hyphal specific gene expression, occurs when the STRE-binding protein Nrg1p, a $(C_2H_2)_2$ Zn finger protein, is inactivated. Since hypha-specific promoters carry STRE elements, Nrg1p might target the Tup1p repressor to hypha-specific promoters via STRE elements. In fact, a similar situation has been demonstrated in *S. cerevisiae*. In *S. cerevisiae* Tup1p acts as a general repressor of several functions, including those controlled by Mig1p, which mediates glucose repression and carries two C_2H_2 Zn finger structural motives. Accordingly, Mig1p could target the general repressor Tup1p to genes related to energy metabolism, just as Nrg1p targets Tup1p to genes that regulate cell response to morphogenesis. In fact, in collaboration with other groups, the Brown laboratory has classified 2000 *C. albicans* genes on the basis of their regulation by Nrg1p, Mig1p or Tup1p by transcript profiling. For example, genes that show >3-fold derepression in the *nrg1* mutant (compared to wild type) were arbitrarily considered as being regulated by Nrg1p. They found that 65 genes out of the 2000 genes were regulated by Nrg1, Tup1 and Mig1. In addition, they found that 35 out of the 2000 genes were regulated by Nrg1 and Tup1. The top 25 Nrg1p-Tup1p co-regulated genes revealed by transcript profiling include the hypha specific genes *ALS3*, *ALS8*, *ECE1*, and *HWP1*, and most Nrg1p-Tup1p co-regulated genes carry the predicted Nrg1p binding site in their promoters. Interestingly, *HYR1* is not included presumably because this gene is only transiently upregulated during hyphal development. Finally, 59 of the 2000 genes were regulated by Mig1p and Tup1p. As expected from the action of Mig1p in the regulation of glucose repressed genes in *S. cerevisiae*, a significant proportion of the Mig1p-Tup1p co-regulated genes in *C. albicans* encode metabolic functions. A conclusion of these studies is that, as expected from the great number of in vitro hyphal inducers, promoters of hypha-specific genes are able to integrate morphogenetic signals with environmental stimuli to regulate hyphal development under appropriate conditions in vivo.

Internal factors that regulate yeast-hypha transition in *C. albicans*, such as polyamines, were analyzed by Ángel Domínguez from the University of Salamanca. Polyamines are basic molecules required for cellular growth and differentiation in many organisms. They stabilize RNA, stimulate DNA and RNA synthesis in vitro and improve translation fidelity. In mammals and fungi, they are made via a pathway initiated by ornithine decarboxylase (ODC) that forms putrescine from ornithine. The level of ODC activity in quiescent cells is extremely low but strongly induced by a wide variety of stimuli; an example is fungal spore germination. These authors have detected a transient increase in ODC activity during the dimorphic switch of *C. albicans*. However, this increase was not accompanied by a parallel rise in the levels of the transcript, suggesting that, during differentiation, ODC activity levels may be regulated post-transcriptionally.

Following isolation of the gene coding ornithine decarboxylase (*CaODC*), they constructed *odc* null mutants that behave as polyamine auxotrophs and grow exclusively in the yeast form at low polyamine levels under all conditions tested. An increase in the levels of external polyamine restored the capacity to switch from the yeast to the filamentous form. In addition to this defect in morphogenesis, the null mutant showed an increased sensitivity to salts and calcofluor white and was avirulent in a mouse model. These results suggest that polyamine levels could exert a pleiotropic effect on transcriptional activity.

Saccharomyces cerevisiae*, a model organism to understand *Candida albicans The similarity between *S. cerevisiae* and *C. albicans* have prompted some researchers to use the former organism to understand observations made in the latter. An example was provided by Judith Berman from the University of Minnesota, USA, who has analyzed the role of *INT1*, a *C. albicans* gene initially cloned on the basis of its hybridization with a human αM integrin probe. Int1p is important for adhesion and virulence, as well as for filamentous growth under most if not all conditions that stimulate hyphal growth. Int1 mutants also have a reduced virulence.

When expressed in *S. cerevisiae*, *INT1* induces formation of highly polarized buds that are similar in appearance to *C. albicans* germ tubes. Cell wall analysis indicates that chitin staining is diffuse and cytokinesis is defective, a phenotype of certain septin mutants. Closer analysis, using septin GFP-fusions, Int1-GFP fusions and indirect immunofluorescence, indicate that septin and Int1p co-localize to a unique pattern of rings and spirals. In addition, Int1p immunoprecipitates with the septin Cdc11p, which is indicative of physical interaction. In *C. albicans*, the Int1-GFP derived from expression of an *INT1-GFP* allele in the *INT1* locus, colocalized to the bud necks in blastospores and pseudohyphae and to the septa of true

hyphae. Accordingly, it seems that Int1p influences *C. albicans* morphogenesis through interactions with septins and possibly other proteins in the bud neck. Int1p carries a C-terminal plekstrin homology domain. A similar domain is found in the C-terminus of Bud4. Deletion of this domain from Int1 and fusion of the remaining protein to GFP resulted in a structure that did bind septins in ectopic structures that were not localized to the periphery of the cell.

A second effect of *INT1* expression in *S. cerevisiae* is the hyperpolarization of the actin cytoskeleton. This effect is reduced in specific *act1* alleles. Moreover, the *INT1*-induced elongated phenotype requires Sla2p, a protein whose C-terminal region is similar to murine talin. One possibility is that Int1p interacts with Sla2p. Although the *ScSLA2* gene is not required for all kinds of polarized growth in *S. cerevisiae*, *sla2/sla2* cells of *C. albicans* are highly defective in the formation of hyphae, even when induced with serum. These findings suggest that Sla2 has a role in germ tube elongation and demonstrates that the study of *INT1* in *S. cerevisiae* can provide new insights into the requirements for *C. albicans* hyphal growth.

Polarization of growth in *S. cerevisiae* is also controlled by some regulatory components of the cell cycle. High levels of the mitotic cyclin Clb2p are associated with isotropic growth during the G2 phase of the cell cycle; thus, *clb2* mutants exhibit polarized growth whereas overexpression of *CLB2* generates extremely round cells. Expression of *CLB2* in *S. cerevisiae* is regulated by two redundant transcriptional repressors, Fkh1/Fkh2. In addition, phosphorylation of Clb2p by the kinase Swe1p regulates the switch from polarized to isotropic growth. Only one homologue of Fkh2 has been found in *C. albicans*. Null mutants lacking *CaFKH2*, or repression of the only copy of *CaFKH2* in heterozygotes, leads to the production of pseudohyphal cells under conditions that favor blastospore formation. A simple hypothesis is that pseudohyphae need Fkh2 to yield either blastospores or hyphae.

In summary, genetic epistasis experiments indicate that Swe1p and Sla2p contribute independently to *INT1*-induced filamentation. Other members of the Sla2-dependent elongation pathway are still to be discovered. In addition, identification of genes regulated by CaFkh2p will be required to understand the regulatory hierarchy of this repressor relative to other well known transcription factors and cell processes that influence *C. albicans* morphogenesis.

Mating in *Candida albicans* The most anticipated lectures were those dealing with the analysis of the *MAT* locus and the preliminary evidence for mating in *C. albicans*. This organism has been considered diploid, and neither mating nor reduction in chromosome number to a haploid condition, i.e. meiosis, has been observed in natural isolates. This contrasts with the well known processes of mating and sporulation described in the closely related yeast *S. cerevisiae*, where both kind of cells, haploid and diploid, can be easily obtained. Christina Hull, from A. D. Johnson's laboratory at the University of California-San Francisco, USA, described the features of the *C. albicans* mating-type-like locus which, like its counterpart in *S. cerevisiae* diploid cells, is heterozygous and encodes the three transcriptional regulators **a**1, α1 and α2. In addition, it contains three additional pairs of genes never seen before in fungal mating-type loci, including poly(A)polymerases, oxysterol binding proteins and phosphatidyl inositol kinases. Since diploid *S. cerevisiae* a/α cells never mate, Hull and coworkers constructed a series of *C. albicans* strains with deletions in the a1 or α1 and α2 genes. In *S. cerevisiae* these kind of strains behave as functional α and **a** strains. In addition, the "unisex" *C. albicans* strains were made *ade2/ade2* and *ura3/ura3* to allow detection of successful mating by selecting Ade^+Ura^+ prototrophs. Early failures to obtain recombinants in vitro prompted them to use a mouse tail vein model of infection. Most of the resulting prototrophic conjugants recovered from the mice contained both markers from each starting strain, were mononucleate and showed increased DNA content, but no hints of meiosis has been detected so far. By contrast, isolation of some conjugants that are trisomic for the *MTL*-containing chromosome locus suggests that the lost of chromosomes could convert the tetraploid strains to the original diploid state.

Beatrice B. Magee, from the University of Minnesota, USA, presented evidence for mating in vitro. During their studies in the *C. albicans* genome project, they had mapped the MTL (mating-type-like) genes to chromosome 5 (Chr 5). Studies by Elena Rustchenko's lab (see below) had shown that adaptation by *C. albicans* for growth on sorbose required the loss of one copy of Chr 5. Accordingly, Magee generated **a** and α strains by growing various *C. albicans* auxotrophic mutants on sorbose and confirmed that those colonies which were monosomic for Chr 5 when analyzed in pulsed-field gel electrophoresis have also lost either the *MTLa* or *MTL*α gene when analyzed by Southern hybridization. The new *MTLa* and *MTL*α strains carrying complementary auxotrophic markers were cross-streaked on YEPD agar plates and the appearance of prototrophs on minimal plate replicas was taken as a signal of successful mating. These prototrophic products contain both *MATa* and *MAT*α, the rDNA genes from both parents and the footprint of the CAI-4 parent. Each marker was present on a different chromosome. Again, the cells were mononucleate, tetraploid in DNA content, and stable. Finally, as with the C. Hull strains, mating required crosses of complementary *MTL* strains, i.e. *MTLa-MTL*α, but failed when *MTLa-MTLa*, *MTL*α*-MTL*α or *MTLa/*α were involved. BB Magee presented pictures showing cell morphologies similar to zygote formation in *S. cerevisiae*.

Non-homologous recombination Germán Larriba presented work from his laboratory (University of Extremadura, Badajoz, Spain) dealing with non-homologous recombination in *C. albicans*, in particular with a DNA ligase (*CaLIG4*). The encoded protein displayed a significant similarity to ligase IV from *S. cerevisiae* and from humans, which are involved in non-homologous end-joining (NHEJ) of DNA double-strand

breaks. This illegitimate recombination process is able to produce novel arrangements of genes, as illustrated by its involvement in the generation of the antigen-binding repertoire in higher eukaryotes. Accordingly, NHEJ could well have evolved to become a prevalent recombination mechanism in an organism unable to create variability by sexual recombination (at least under most circumstances), such as *C. albicans.* In addition, since irradiation and anticancer formulations given to cancer patients also reach *C. albicans* cells, it is important to know how fungal cells respond to DNA damaging agents and the pathways they use to repair double-strand breaks accumulated in their DNA.

A *CaLIG4Calig4* heterozygote showed a pronounced defect in myceliation which was slightly increased in the null strain. Reintroduction of the wild-type allele in the null mutant partially restored the myceliation capacity of the cells. In agreement with the positive role of *CaLIG4* in morphogenesis, a significant rise in mRNA levels has been detected during the morphological transition. Accordingly, the NHEJ apparatus of *C. albicans* may control morphogenesis in this diploid organism. In addition, deletion of one or both copies of *LIG4* resulted in an attenuation of virulence in a murine model of candidiasis.

CaLIG4 is not essential for DNA replication nor for the repair of DNA damage induced by ionizing radiation or UV light, indicating that these lesions are repaired primarily by homologous recombination. Recent results in mammals indicate that *LIG4* is a crucial caretaker of the integrity of the genome. However, preliminary studies have indicated that null *lig4* mutants do not show spontaneous chromosomal rearrangements. Still, chromosome R exhibits marked changes during the generation of single and double *lig4*, but not other open reading frames. Further studies under stress conditions may reveal the importance of NHEJ in *C. albicans.*

Chromosomal rearrangements Finally, Elena Rustchenko, from the University of Rochester, USA, discussed chromosomal rearrangements, including deletions, translocations and aneuploidies, that occur when *C. albicans* cells are grown under a variety of conditions. Her group has proposed that chromosomal instability is a way in which cells acquire genetic variability in the absence of the more conventional method, i.e., meiotic segregation. In fact, one type of genetic alteration, i.e., nondisjunction, resulting in altered chromosome copy number of different specific chromosomes, has been related to the appearance of specific mutants. For instance, mutants able to grow on sorbose, are monosomic for Chr 5. This results in the activation of a structural gene *SOU1* located in a different chromosome (Chr 4). Duplication of the remaining homologue makes cells regain the original Sou- phenotype through down-regulation of *SOU1*. Adaptation to sorbose is not the only example reported so far. There are a number of cases in which chromosomal nondisjunction controls important functions, such as utilization of different nutrients or resistance to the antibiotic fluconazole. Trisomy increases the copy number of all genes located on a chromosome, whereas monosomy implies the existence of negative regulators. Thus, it is suggested that *C. albicans* contains a resource of potentially beneficial genes, as well as their positive and negative regulators, which are distributed over chromosomes in such a way that their expression can be activated by changes in chromosome number. In other words, changes in chromosome number determine the ratio of regulatory/structural genes and accordingly the expression of the latter. In fact, cloning of negative regulators that mediate sorbose utilization is currently being accomplished. Some of them reside on Chr 5 (*CSU51*,*CSU52*, etc), whereas others are distributed over several chromosomes and represent different levels of cellular inhibition of genes for the secondary carbon sources. One of the latter, *CSU3* has been shown to affect *SOU1* at transcriptional level similar to Chr 5 monosomy, but does not influence the frequency of Chr 5 alteration. On the other hand, *CSU51*, whose levels are, as expected, affected by Chr 5 copy number, could serve as an "override", thus allowing ready adaptation to environmental changes. These studies may be linked to these reported by Larriba's group. The fact that mammalian cells deficient in *LIG4* show frequent chromosomal anomalies, including chromosome breaks and aneuploidies, suggest that *LIG4*, through NHEJ, is an attractive candidate involved in the gene regulation controlled by chromosome copy number.

We hope that the interest raised by the topics presented in this symposium create new insights that will accelerate our knowledge of *C. albicans* biology. This research may provide a necessary step towards the development of new, faster and more precise diagnosis tests and drugs for treatment of candidiasis.

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