

The Role of Glutamine-Rich Region of *Candida Albicans* Tec1p in Mediating Morphological Transition and Invasive Growth

W. Abu Rayyan, A. Singh, A. M. Al-Jaafreh, W. Abu Dayyih, M. Bustami, S. Salem, N. Seder, K. Schröppel

Abstract—Hyphal growth and the transcriptional regulation to the host environment are key issues during the pathogenesis of *C. albicans*. Tec1p is the *C. albicans* homolog of a TEA transcription factor family, which share a conserved DNA-binding TEA domain in their N-terminal. In order to define a structure-function relationship of the *C. albicans* Tec1p protein, we constructed several mutations on the N terminal, C terminal or in the TEA binding domain itself by homologous recombination technology. The modifications in the open reading frame of TEC1 were tested for reconstitution of the morphogenetic development of the *tec1/tec1* mutant strain CaAS12. Mutation in the TEA consensus sequence did not confer transition to hyphae whereas the reconstitution of the full-length Tec1p has reconstituted hyphal development. A deletion in one of glutamine-rich regions either in the Tec1p N-terminal or the C-terminal in regions of 53-212 or 637-744 aa, respectively, did not restore morphological development in mutant CaAS12 strain. Whereas, the reconstitution with Tec1p mutants other than the glutamate-rich region has restored the morphogenetic switch. Additionally, the deletion of the glutamine-rich region has attenuated the invasive growth and the heat shock resistance of *C. albicans*. In conclusion, we show that a glutamine-rich region of Tec1p is essential for the hyphal development and mediating adaptation to the host environment of *C. albicans*.

Keywords—*Candida albicans*, transcription factor, TEA domain, hyphal formation, morphogenetic development, TEC1, Tet-induced.

I. INTRODUCTION

THE TEA transcription factors family comprises regulatory proteins which control the temporal and spatial expression of stage-specific genes involved in the development and morphogenesis of different organisms [1]. Their members share an N-terminal TEA domain which interacts with a TEA consensus sequence (TCS) in the target promoters [2]. The

Walid Abu Rayyan is with the Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan and University of Petra Pharmaceutical Centre (UPPC), University of Petra, Amman, Jordan (corresponding author, phone: 00962-790272152, fax: 00962-6-4291432, e-mail: walid.aburayyan@uop.edu.jo).

Anurag Singh is with University Children's Hospital, Pediatric Infectious Diseases, Immunology & Pneumology/Cystic fibrosis. Tübingen, Germany.

Ahmad M. Al-Jaafreh is with Faculty of Science, Department of Medical Laboratory Sciences, Mutah University, Mutah, Jordan.

Wael Abu Dayyih and Mona Bustami are with the Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

Shadi Salem is with the Department of Basic Sciences and Humanities, Imam Abdulrahman Bin Faisal University, Dammam, KSA.

Nisreen Seder is with the School of Biomedical Science, Faculty of Health Sciences, University Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia.

Klaus Schröppel is with The Institut für Medizinhygiene. Tübingen, Germany.

TEA homolog Tec1p of the human fungal pathogen *Candida albicans* is essential for regulation of gene expression and morphogenetic development [3]-[5]. Regulation of biofilm formation also involved Tec1p, which in this case is mediated through up-regulating the zinc -finger protein Bcr1p, activating the expression of the adhesion proteins ALS3p and HWP1p [6]. This promotes the adhesion of *C. albicans* to the host epithelial tissues during colonization and pathogenesis [7], [8]. Finally, virulence of *C. albicans* is dependent on the expression of *TEC1* gene in a model of experimental candidiasis and also adaptation of the fungal cell to the mammalian environment are considered to be crucial steps during pathogenesis [5], [9].

For instance, in the ancestor of *C. albicans*, it was suggested that differential regulation of haploid invasive and diploid pseudohyphal growth of *S. cerevisiae* by Ste12p and Tec1p is not only under combinatorial control but also requires additional mechanisms. The authors suggested that Ste12p activated *TEC1* expression via clustered PREs and Tec1p regulated expression of target genes, e.g., *FLO11*, by TCS control [10], [11].

On the other hand, Tec1p induced development of *S. cerevisiae* in the absence of Ste12p, a terminal regulator of the pheromone response pathway [12]. The TCS elements for DNA binding of Tec1p were sufficient to allow for Tec1p-driven gene expression by a mechanism termed TCS control which was operative in the absence of Ste12p. TCS control, *FLO11* expression, and haploid invasive growth required the C-terminus of Tec1p. In contrast, the Ste12p-dependent FRE control mechanism required the N-terminal portion of Tec1p, which contains the TEA/ATTS DNA-binding domain [5], [13].

The exact mechanisms of Tec1p-dependent upregulation of genes and physical interactions with other regulators are poorly understood in *C. albicans*. Although it was reported that induction of cell-surface genes and biofilm formation required Tec1p for the expression of Bcr1p, but so far the mode of action of the regulatory pathway was not elucidated [14], [8].

Both *C. albicans* and *S. cerevisiae* place Tec1p in a crucial signal transduction pathway, and since the *C. albicans* Tec1p is much longer than its homolog in *S. cerevisiae*, we investigated whether the *C. albicans* specific region of Tec1p contains a putative protein domain which might be differentially required either for DNA binding or for cooperative interaction with DNA or proteins, respectively.

In this study to minimize the influence of multiple environmental factors, we integrated modified (open reading frames) ORFs of *TEC1* which were under the control of the *C. albicans* -adapted reverse tetracycline (Tet)-dependent transactivator (rtTA) and used Tet-induced morphogenetic development, and nematode infection as a read-out for complete restoration of Tec1p activity.

As well we expected that the C-terminal part of Tec1p exerts an essential function very early during Tec1p-dependent signal transduction after physical interaction of the TEA domain with TCS, we predicted that modified ORF of *TEC1* can be generated which encode DNA binding proteins without biological activity.

As a consequence of rational thinking, we assumed a direct relationship between the structure and the function of different regulatory regions of Tec1p not only in the previously known TEA domain itself but also in the possible N or C-terminal motifs of Tec1p. This can also help us to solve the mystery of possible putative functional domains in the *TEC1* protein. We believe that due to the high degree of homology between the TEA binding domain structures of *C. albicans* and *S. cerevisiae*, also the similarities shared in mediating the pheromone response between the two ancestors and the previously reported importance of the C-terminal motif of *C. albicans* in the regulation of signal transduction [15,6], we have addressed the question whether the extra length of 261 aa in the C-terminal region of *C. albicans* Tec1p than its Tec1p homolog in *S. cerevisiae* has a regulatory role in the gene activation and post-translational modifications in the protein.

II. MATERIALS AND METHODS

A. Strains and Growth Conditions

C. albicans strains used in this study are listed in Table I. All strains were stored as frozen stocks in 35 % glycerol at -80 °C. Strains were routinely grown in YPD medium at 25 °C, for growing on plates, 1.5 % agar was added to the medium. For the selection of nourseothricin-resistant (Nou^R) transformants, 200 µg/ml of nourseothricin (Jena, Bioscience) was added to YPD before pouring the agar plates. For morphological experiments in liquid cultures, the strains CWR01-CWR06 were inoculated at low density into DMEM (Dulbecco's Eagle Medium) containing 40 mM HEPES buffer in the presence or the absence of Tet at 25°C for 24 hours.

B. Construction of Plasmids and Transformation of *C. albicans*

Mutants of wild-type *TEC1* open reading frame (ORF) were constructed and synthesized by PCR amplification and subcloned into pGEM-T-easy as outlined in Table II. Inserts were further subcloned to replace the *SaII*/*BglII* inserts of pNIM1 [16] generating a library of pNIM1 subclones were the modified *TEC1* ORFs ORF was placed behind a Tet-inducible promoter (Table II).

The *SacII* / *Apal* fragments of the pNIM1 subclones were transformed into CaAS12, targeting the *ADHI* promoter, yielding strains CWR01, CWR02, CWR03, CWR04, CWR05, and CWR06 (Table I).

C. Southern Blot

Proper integration of DNA fragments was confirmed by Southern blot analysis. Strains were cultivated in YPD medium overnight, harvested and subjected to cell wall digestion by the addition of 300 µg/ml Zymolase at 37°C for 18 hrs. Genomic DNA was extracted using a commercial kit according to the instructions of the manufacturer (Promega). Genomic DNA was digested with *EcoRI* for 16h at 37°C, and loaded on 0.7% agarose gels in 0.5% TBE buffer at 30 V for 4h. After that, gels were treated with 0.2 M HCl for 20 min at room temperature and soaked in 0.5 M NaOH-1.5 M NaCl for 60 min to denature the DNA. Capillary blot transfer of the DNA fragment onto Biodyne B nylon membrane (Pall, Dreieich) was followed by subsequent hybridization using a digoxin UTP hybridization kit (Roche, Germany). The pNIM1 plasmid was used to prepare *ADHI* probe by digesting it with the *Sac II* and *XbaI* restriction enzymes. The DNA fragments were separated by gel electrophoresis and the *ADHI* probe fragment of 700 bp was purified from the gel by standard procedure.

D. RT-PCR

An overnight culture of *C. albicans* was inoculated in YPD medium at 25°C. Cells were harvested at OD₆₀₀ of 1 and then further incubated in DMEM medium supplemented with or without doxycycline at 25°C for 60 min. Total RNA was extracted according to standard procedures. The quality and concentration of the RNA samples were examined by gel electrophoresis and measured spectrophotometrically at 260 nm, respectively. Using total RNA isolated from *TEC1* ORF mutant strains, cDNA was synthesized using the *Superscript III first-strand synthesis super mix* for qRT-PCR (Invitrogen) according to the manufacturer's instructions. PCR reactions were performed in a total reaction volume of 25 µl. Primer pairs used for amplification are listed in Table III.

E. Virulence Assays in a Nematode Infection Model

Wild-type *C. elegans* strain N2 was used as an infection model host. Worms were propagated on nematode growth medium (NGM) supplemented with of *E. coli* OP 50 lawns as a food source [17]. For infection, *C. albicans* strains wild-type SC5314, *tec1* mutant CaAS12, CWR01, CWR02, CWR03, CWR04, CWR05, and CWR06 were used. *Candida* lawns were prepared on NGM plates by spreading fully grown overnight culture in YPD media and plates were incubated for 24 hr at 25°C. Synchronized L4 larvae of *C. elegans* were allowed to crawl and feed on *C. albicans* lawn for four hours [18]. Worms were washed with sterile M9 buffer and transferred to S media in presence and absence of 50 µg/ml doxycycline and incubated at 25°C. Plates were observed at regular intervals to monitor nematode infection, survival and hyphal formation by *Candida* strains.

III. RESULTS

A. Morphological Development of Functional *TEC1* Mutants

The *TEC1* gene contains an ORF of 2,232 nucleotides (nt)

[19], which harbors several structural elements including a DNA binding TEA domain, glutamine and cysteine-rich regions as depicted in Fig. 1.

We introduced several mutations which spanned over the TEA domain, or sequences close to the 5' or 3' end of the *TEC1* ORF (Fig. 1) and integrated each deletion construct as well as a full-length control construct at the *ADHI* locus of the *tec1/tec1* mutant CaAS12 (Fig. 2) [5], proper integration was confirmed using Southern blotting (Fig. 3). The expression of the recombined ORF was induced by the addition of tetracycline to the culture media.

As a test for the expression and proof of principle, we cultivated the wild-type strain SC5314, the parental strain CaAS12, the full length control strain CWR06 and the TEA domain deletion negative control strain CWR03 in the absence or the presence of 50 µg/ml of tetracycline at 25°C, which favoured growth of the yeast form of *C. albicans* (Fig. 4).

Morphogenetic development was only induced in strain CWR06 because it contained a Tet-responsive *TEC1* ORF. The other strains did not respond to Tet and continued to replicate as yeast cells. This confirmed that the Tet-induced expression of an only functional *TEC1* ORF as in CWR06 would reintroduce the capacity for morphogenetic

development in the CaAS12 *tec1/tec1* mutant background but not in CWR03, which is deleted for the TEA domain.

The strains CWR01, CWR02, CWR04, and CWR05 were replicating in the yeast form when incubated at 25°C in DMEM. Whereas, when tetracycline was added to the media, morphological development was induced in strains CWR01, CWR02, while CWR04 and CWR05 continued to grow in the yeast form. This result was stable until the cultures were finished after 72 hours (Fig. 4).

The construction of modified *TEC1* ORFs (gray boxes) was conducted by amplifying the ORFs from a DNA library using for. *SalI* and *Rev. BamHI* primers ligated behind a tetracycline-inducible promoter (Tet promoter) in the *TEC1* recombination cassette. CWR01, CWR02, CWR05, CWR09, CWR11, and CWR12 strains harbor *TEC1* ORFs that share a deletion in the C-terminal region highlighted in the dotted box. The CWR04 strain has an N-terminus deletion. The CWR06 strain contains the wildtype *TEC1* ORF and CWR03 do not contain the TEA DNA binding domain (black box). The regions of *TEC1* with the distinctive amino acid composition are indicated by the hatched boxes. The numbers indicate the nucleotide base pair number

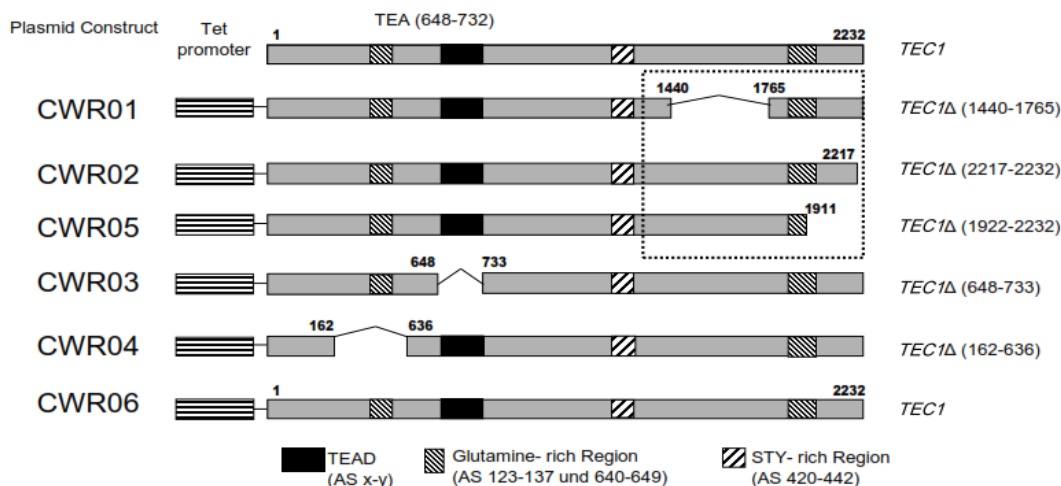


Fig. 1 Schematic diagram of *TEC1* ORF mutants

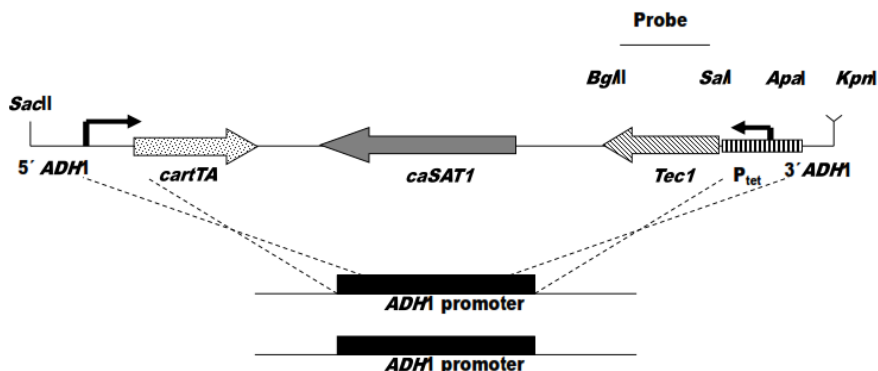


Fig. 2 Cloning of Recombination cassette of *TEC1* into *ADHI* locus of CaAS12

The recombination cassette of *TEC1*. Ptet: Tetracycline promoter. *ADH1*: *ADH1* promoter of *C. albicans*. *CaSAT1*: dominant selection marker nourseothricin. *carT1*: *C. albicans*-

adapted versions of heterologous genes. *ApaI*, *BglII*; *SacII*; *SalI*, *ApaI*, *KmpI* unique restriction sites.

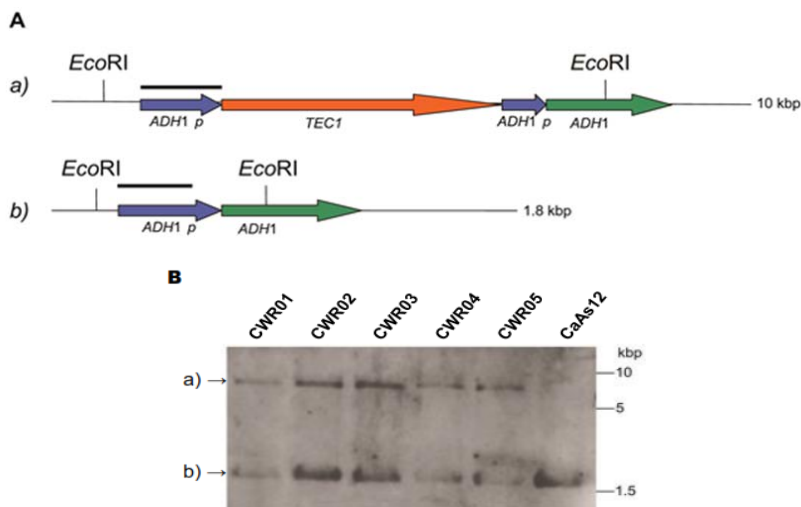


Fig. 3 Southern blot analysis of the revertant strains. A -Schematic structures of *ADH1* locus of the revertant strains. a) The structure of the *TEC1* recombination cassette integrated into the *ADH1* locus, b) The structure of the wild-type *ADH1* locus. B- Genomic DNA of the revertant strains and knockout *tec1/tec1* CaAS12 strains was digested with *EcoRI* and hybridized with *ADH1p* (the lined fragment) (0, 95 kb using *SacII* *XbaI* from pNIM1). Two bands were revealed a) the first allele harbors the integration cassette 8 Kbps, b) 1.8 Kbp band of the wild-type *ADH1* locus band. "Gene Ruler 1kp plus DNA Ladder" Fermentas was used as size marker

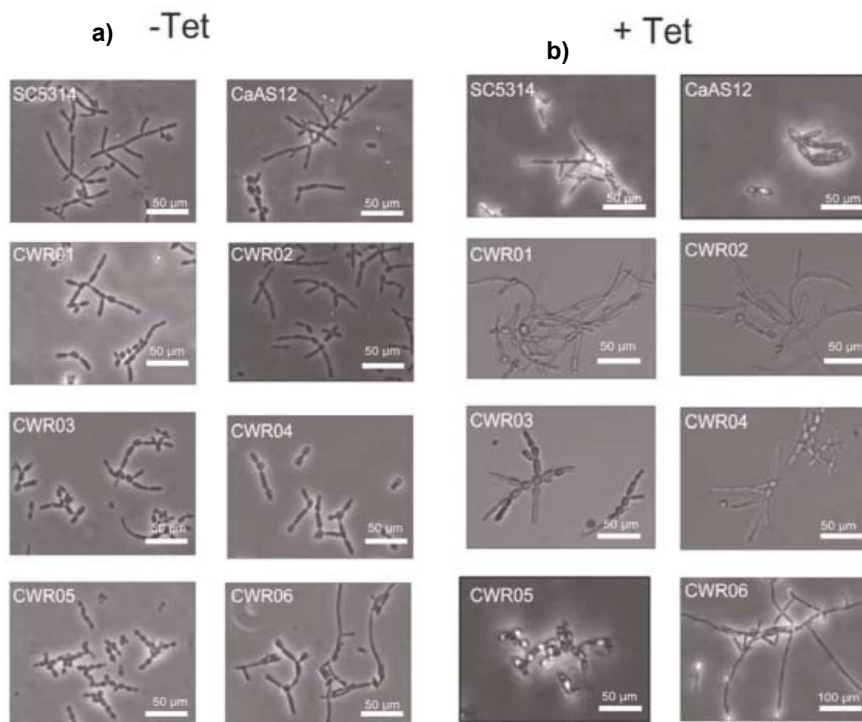


Fig. 4 Morphological development of the revertant strains in liquid medium. *C. albicans* cells were cultured in DMEM 40mM HEPES 5% CO_2 at 25°C for 8 h. a) Morphology of candida strains without *TEC1* expression no doxycycline was added. B) The *TEC1* expression was induced by adding 50μg/ml doxycycline (+Tet). Wildtype SC5314, CaAS12 *tec1/tec1* mutant, revertants CWR03, CWR04, as well as CWR05 cells were unable to develop true hyphae morphology and they grow as pseudohyphae cells. The full length revertant CWR06 strain showed morphogenetic switch to true hyphae, and the C-terminal mutants CWR01 and CWR02 were also able to show a poly morphogenetic appearance of pseudohyphae and true hyphae under the expression of a *TEC1* transcription factor

B. Pathogenesis of Reverent TEC1 Strains in vivo Model C. elegans

Wild-type *C. elegans* N2 was infected with *C. albicans* by exposure to the blastoconidia for four hrs on NGM agar plates, and after transfer to S-media, host nematodes were incubated for 5 days at 25°C. WT SC5314, full-length revertant CWR06, and C-terminal mutants CWR01 and CWR02 affected the behavior of *C. elegans* and were able not only to invade the host but even to penetrate the surface cell layer of the nematodes before they could be detected by microscopic examination outside of *C. elegans* (Figs. 5 and 6). On the other hand, CaAS12 *tec1/tec1* mutant, as well as revertants the CWR03, CWR04, and CWR05 were unable to form hyphae after exposure to the worms. Infected populations of *C. elegans* were scored for survival at regular intervals. We found that killing of the worms occurred only after the hyphal

invasion of the host. Initial hyphae could be detected as soon as 24 hrs after infection and all dead worms showed an obvious penetration of the cuticle with hyphae. *C. albicans* mutants which were not capable of morphogenetic development caused less number of dead worms in comparison to *C. albicans* mutants which could form hyphae (Figs. 5 and 6). CWR05 caused the least dead worms amongst all the strains, suggesting that its lack of hyphal formation prevented the successful invasion of this strain from the gastrointestinal tract into the tissues of *C. elegans*. Interestingly, *C. albicans* cells formed hyphae at 25°C only after getting ingested by the worm and being exposed to the worm's intestinal environment, whereas *C. albicans* cells remaining outside the worm showed no hyphal development and maintained the yeast form of growth.

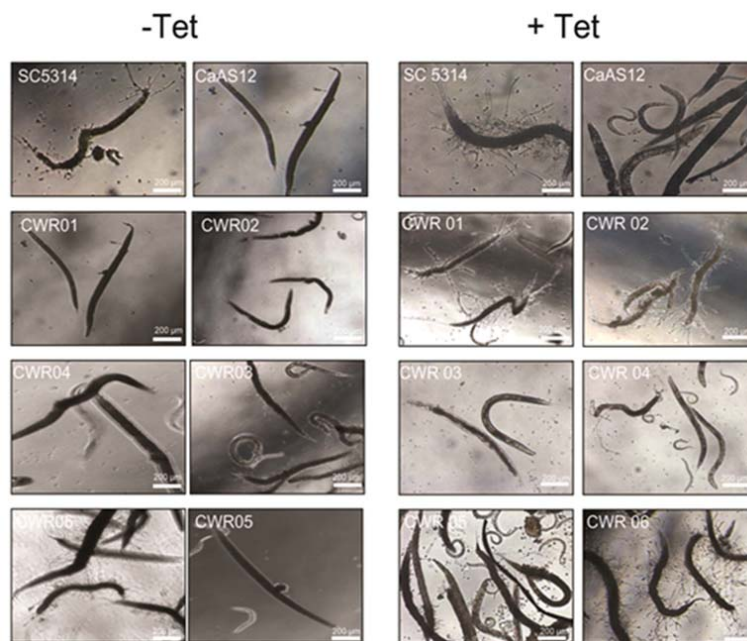


Fig. 5 *C. elegans* N2 infected with revertant strains. Wildtype *C. elegans* N2 infected with various *C. albicans* strains. CaAS12 *tec1/tec1* mutant, revertants CWR03, CWR04, and CWR05 strains were unable to form hyphae after infecting worms. Worms were exposed for four hrs to *C. albicans* grown as a lawn on NGM agar plates and incubated for 5 days at 25°C in S-media for *C. elegans*. On the other hand, WT SC5314, full-length revertant CWR06, and C-terminal mutants CWR01, CWR02 are able to show hyphae penetrating through the cuticle of worms after infection. Worms were grown in the same conditions as in the figure. All images were captured in good plates using an inverted microscope (ZEISS, AXIOn 4.4).

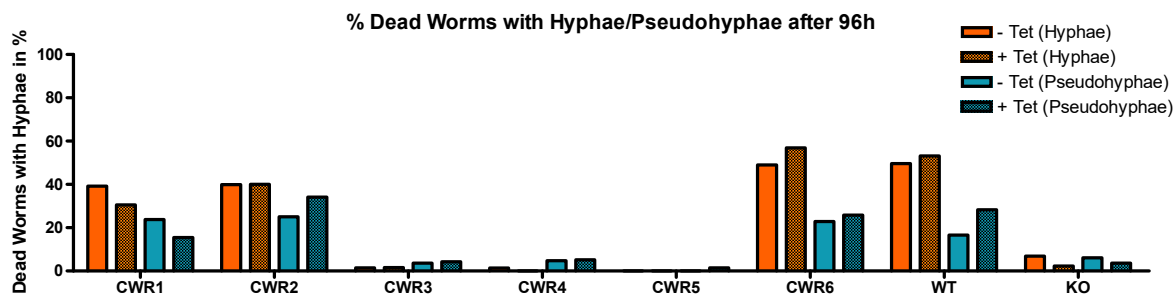


Fig. 6 Virulence of revertant strains. CWR06 and wildtype strains showed high virulence and killing rate in infected words. Glutamine region (CWR04, CWR05) and TEA/ ATTS mutants (CWR03) showed weak virulence and almost no killing rate

IV. DISCUSSION

A. Morphological Development of the Structure-Function Relationship of Tec1p

Transcriptional regulation combined with DNA binding controls the genetic flow from DNA to proteins; ectopic expression of Tec1p from a Tet promoter has restored the abrogation of morphogenetic development in the *tecl/tecl* background. We excluded lacking mRNA expression as a potential reason for the observed non-responsive state for *tecl* truncated strains after tetracycline addition by running RT-PCR for the mentioned strains. We could not detect any difference in the expression levels of the integrated ORF between the TEA mutant strain CWR03 and the full-length strain CWR06 (data not shown), indicating a tetracycline-dependent gene expression of Tec1p. Morphological studies on C-terminal truncated Tec1p strains CWR01 and CWR02 showed no obsessive effect for a Tec1p mutation on genetic regulation especially the hyphal length and architecture were similar to the hyphae developed by the full-length Tec1p CWR06 revertant strain. Moreover, we found there were low levels of expression for the ORF of Tec1p even without the addition of the promoter-regulator tetracycline (data not shown). These levels could be explained by the presence of a leaky promoter which sustains the Tec1p expression even in absence of the tetracycline inducer but nevertheless, these levels are not sufficient to induce a morphogenetic transition in revertant strains [12].

Meanwhile, the mutant strain CWR03 which harbors the deletion of TEA domain of *TEC1* ORF showed abrogated yeast to hyphal morphological development in all inducing conditions even after adding tetracycline. The deficiency in DNA binding to the sequence 5'-CATTCC-3' confers a loss in the biological function, which in turn confirms the crucial role of the TCS as a Tec1p recognition sequence in DNA binding and for the transcription activation of the down-regulated genes [20].

In addition to the general influence and impact of the sequence specificity of the protein structures on the DNA binding, it is also known that the stereochemistry of the protein and the 3D structure plays a major role in defining the function of the protein in any physiological process [2]. However, mutations in the glutamine-rich N-terminal of Tec1p of CWR04 or the glutamine-rich C-terminal of Tec1p of CWR05 strains has revealed a complete loss of exerting the biological function of Tec1p and showed abrogation in hyphal transform after the induction of *TEC1* expression by adding tetracycline as the candida strains continued to grow in yeast form. Whereas, the revertant strains CWR01 and CWR02 which harbor mutations outer the glutamine-rich regions have restored the abrogated phenotype and grow in hyphae form. The previous observation points toward not only the TEA domain of the N-terminal is essential for exerting the biological function of Tec1p but also the glutamine-rich regions may intermediate the activation mechanism by forming an activation complex or even a binding site for unknown protein which highlights the presence of a possible

activating domains harbored in the glutamine-rich region of Tec1p. In a similar manner, Hwang et al, 1993 have described a relevant effect for the TEA family member of GAL4-TEF-1 where an activation complex is formed to exert the morphogenetic development [21]. In *S. cerevisiae*, the activation of pheromone response elements (PRE) is mediated through the N-terminal of Tec1p whereas the TCS control, *FLO11* expression, and haploid invasive growth requires the C-terminal of Tec1p [5].

Our studies speculate the involvement of the glutamine N-terminal domain of Tec1p in the DNA binding whereas the activation domain is harbored in the glutamine C-terminal of Tec1p. It has been found that increasing the truncation of the *TEF1* protein caused initially the loss of DNA binding where the binding capacity was abolished in the complete deletion of the C-terminal region of TEAD [2]. In comparison to Tec1p, TEF-1 has a larger N-terminal region. This emphasizes and characterizes the mediation of morphogenetic development in *C. albicans* is not only exerted through the full-length Tec1p; rather, the signal transduction pathway mediates its signals through distinctive regions spanned over the C-terminus of Tec1p [2]. This may give an explanation for the lost phenotype of the deleted glutamine-rich region in both CWR04 (5' end) and CWR05 (3' end) which substantially interfered with the function of Tec1p.

Mutations in the TEA DNA binding domain, as well as glutamine-rich regions of Tec1p, have conferred a yeast growth similar to the *tecl/tecl* knockout strain despite the constituent expression of Tec1p. Such morphology could be contributed to a non-functional copy of Tec1p transcription factor which is not able to activate the expression of downregulated genes. Substantially, Tec1p directly activates the expression of hyphae specific genes (HSG) by binding to the TCS sequences in the gene promoter region [22] and in our case there was no morphogenetic transition pointing to no expression of HSG.

- By blasting the *TEC1* sequence against the prosite database, a matching was found in the region of 1978-2029 aa as a VWFc domain signature was found in the C-terminal glutamine-rich region. It has been demonstrated the most of VWA-containing proteins are positioned extracellularly. Although the ancient proteins harboring the domain were precisely found in eukaryotes, they were involved in intracellular functions such as gene regulation, proofreading, and protein synthesis. Interestingly, the domain is exerting its function through multiprotein complexes. Functions of cell adhesion, dissemination, biofilm formation, and signaling pathways conduction participate in the interaction of large array ligands [23].

In order to test the virulence of our strains under study, we choose the currently described model of *C. albicans* infection in the nematode host *C. elegans*. It combines the advantages of a short observation period, a fast-growing host and is validated for a relevant number of *C. albicans* mutants. During candidiasis infection, *C. albicans* transits between different morphological forms, each form is important to conduct a

specific stage function, for example, the yeast form is important for *C. albicans* disseminating through the bloodstream and to establish the colonization whereas the hyphal form is required to penetrate the lumen of GI tract or to evade from neutrophils and macrophages in the tissues invasion process [24]. The development of each morphological form is combined with a release of the stage-specific genes, among them, the adhesion ALS3 and Hwp1 proteins which are expressed mainly in the yeast form to facilitate the adhesion to various surfaces during the colonization process [25], whereas, the Sap 4-6 proteins family are secreted in the hyphal form to lysis the protein constituting the architecture of the host cell wall. To elucidate the influence of Tec1p deletion on the proteases proteins we have used the *C. elegans* as a hosted model and the outbreak of the cuticle as a readout for the gene expression [26].

The effect of Tec1p expression is abundantly clear on the C-terminal mutant CWR01, and CWR02 strains, the full *TEC1* ORF revertant strain CWR06 and the W.T SC5314 in terms of the filamentous elements breaking through the cuticle of *C. elegans* as shown in Fig. 7. These results elucidate that all of the above-mentioned strains express a functional copy of Tec1p which in turn activates a set of hyphae-specific and virulence genes [27]. The aspartyl proteases SAP4-6 isogenes harbor repetitive TEA/ATTS consensus sequences motifs in their promoter region. The expression of SAP4-6 genes is directly mediated through Tec1p activity, and they are strictly secreted in the hyphal form [5]. *C. albicans* mediates the secretion of the proteases proteins in order to penetrate the tissues layers of the host or even to evade from the proteolytic activity of macrophages. On the other hand, we observed almost no hyphae outbreak in the CaAS12, CWR03, CWR04 and CWR05 strains which can be included as an expression of a non-functional copy of Tec1p, either for lacking the activity of gene expression in CWR04 and CWR05 strains or due to a

loss in DNA binding activity in CWR03 strain or even due to a lack of the homologous copies of *tec1* in CaAS12. We can figure out from this observation that not only the TEA domain of Tec1p is important for the Sap 4-6 expression but also the C-terminal region has a role in mediating the protein expression. Interestingly, we observe the same morphogenetic development pattern for the revertant cells in the response to different incubation conditions as the strains which showed morphological development in liquid medium showed also the filamentous outbreak from *in vivo* model. This highlights the important role of the glutamine-rich region of Tec1p in controlling and mediating different biological functions in *C. albicans*.

The proposed structure-function relationship mediated by Tec1p transcription factor

In conclusion, we propose the following schematic diagram (Fig. 7) for the important functional regions of Tec1p and we focus on the CHAM domain.

The transcription factor Tec1p harbors 744 aa and contains four distinct structural regions. Our mutational studies have revealed the importance of three functional regions of them in mediating biological activity of Tec1p and they are summarized as follow:

- 1- The N-terminal glutamine-rich regions I between 7 and 172 aa
- 2- The N-terminal TEA DNA binding domain region II between 216 and 244 aa.

Both regions have distinct structures for DNA binding activity of Tec1p.

- 3- The C-terminal region III between 637 and 709 aa which we proposed name CHAM (C-terminal Hyphal activation Motif) for it, is required for mediating biological functions of morphological development, *in vivo* pathogenesis, invasive growth on solid agar.



Fig. 7 Schematic diagram of distinct functional regions of Tec1p. The regions I and II in the N-terminus are required for DNA binding. The C-terminus CHAM region required for gene activation

TABLE I
WILDTYPE AND MUTANT STRAINS USED

Strain or plasmid	Origin	Relevant genotype or characteristics ^a	Reference or source
SC5314		the wild-type strain	Gillum <i>et al.</i> , 1984 [28]
CaAS12	SC5314	<i>Tec1A::hisG / tec1A::hisG-URA3-hisG</i>	Gillum AM, <i>et al.</i> , 1984 [28]
CWR01	CaAs12	<i>ADH1/adh1 :: pTetTEC1A (1440-1765)</i>	This study
CWR02	CaAs12	<i>ADH1/adh1 :: pTetTEC1A (2217-2232)</i>	This study
CWR03	CaAs12	<i>ADH1/adh1 :: pTetTEC1A (648-733)</i>	This study
CWR04	CaAs12	<i>ADH1/adh1 :: pTetTEC1A (162-636)</i>	This study
CWR05	CaAs12	<i>ADH1/adh1 :: pTetTEC1A (1922-2232)</i>	This study
CWR06	CaAs12	<i>ADH1/adh1 :: pTetTEC1</i>	This study

TABLE II
VECTORS USED TO CONSTRUCT MUTANT STRAINS

Strain or plasmid	Origin	Relevant genotype or characteristics ^a	Reference or source
pBAIT Vector			
p391	Sc5314	<i>TEC1</i>	Gillum <i>et al.</i> , 1984 [28]
p428	p391	<i>TEC1A</i> (21-517)	Sehna, 2008 [29]
p433	p391	<i>TEC1A</i> (1922-2232)	Sehna, 2008 [29]
p434	p391	<i>TEC1Δ</i> (1440-1765)	Sehna, 2008 [29]
p446	p391	<i>TEC1Δ</i> (2217-2232)	Sehna, 2008 [29]
p492	p391	<i>TEC1Δ</i> (648-733)	Sehna, 2008 [29]
pGEM-T Easy II			
p499	p428	PCR product <i>TEC1A</i> (162-636) into pGEM-T Easy II	This study
p500	p446	PCR product <i>TEC1A</i> (2217-2232) into pGEM-T Easy II	This study
p501	p434	PCR product <i>TEC1A</i> (1440-1765) into pGEM-T Easy II	This study
p502	p492	PCR product <i>TEC1A</i> (648-733) into pGEM-T Easy II	This study
p503	p499	PCR product <i>TEC1A</i> (162-636) in <i>bgII</i> <i>SalI</i> of pNIM1	This study
p504	p500	<i>TEC1A</i> (2217-2232) in <i>bgII</i> <i>SalI</i> of pNIM1	This study
p505	p501	<i>TEC1A</i> (1440-1765) in <i>bgII</i> <i>SalI</i> of pNIM1	This study
p506	p502	<i>TEC1A</i> (648-733) in <i>bgII</i> <i>SalI</i> of pNIM1	This study
p507	p391	PCR product <i>TEC1</i> into pGEM-T Easy II	This study
p508	p507	<i>TEC1</i> in <i>bgII</i> <i>SalI</i> of pNIM1	This study
p509	p433	PCR product <i>TEC1A</i> (1922-2232) into pGEM-T Easy II	This study
p510	p509	<i>TEC1A</i> (1922-2232) in <i>bgII</i> <i>SalI</i> of pNIM1	This study
pNIM			

TABLE III
PRIMERS

primer	sequence
TEC1_F_Sal	5'TGGTCGACATGATGTGCGCAAGCTACTCCTAGT3'
TEC1_R_Bam	5'GGATCCTAAAACACTACTAGTAAATCCTTCTGTGTAT3'

ACKNOWLEDGMENT

We would like to express our appreciation to the directors, physicians, laboratory, and nursing staff of the Institute of medical microbiology and hygiene in Tübingen, Germany. We would further like to thank Kerstin Fischer, for her support in morphogenetic testing and the general assistance. This project was funded by German Ministry for Science and Education (BMBF). Additionally, we like to thank the Deanship of Scientific Research (University of Petra) for supporting the project and the University of Petra Pharmaceutical Centre for providing the infrastructure for conducting the experiments.

REFERENCES

- Freire F, de Barros PP, Pereira CA, Junqueira JC, Jorge AOC (2018) Photodynamic inactivation in the expression of the *Candida albicans* genes ALS3, HWP1, BCR1, TEC1, CPH1, and EFG1 in biofilms. *Lasers in medical science*. doi:10.1007/s10103-018-2487-8.
- Anbanandam A, Albarado DC, Nguyen CT, Halder G, Gao X, Veeraraghavan S (2006) Insights into transcription enhancer factor 1 (TEF-1) activity from the solution structure of the TEA domain. *Proceedings of the National Academy of Sciences of the United States of America* 103 (46):17225-17230. doi:0607171103 (pii) 10.1073/pnas.0607171103.
- Bruckner S, Kern S, Birke R, Saugar I, Ulrich HD, Mosch HU (2011) The TEA transcription factor Tec1 links TOR and MAPK pathways to coordinate yeast development. *Genetics* 189 (2):479-494. doi:10.1534/genetics.111.133629.
- Shareck J, Nantel A, Belhumeur P (2011) Conjugated linoleic acid inhibits hyphal growth in *Candida albicans* by modulating Ras1p cellular levels and downregulating TEC1 expression. *Eukaryotic cell* 10 (4):565-577. doi:10.1128/EC.00305-10.
- Schweizer A, Rupp S, Taylor BN, Rollinghoff M, Schroppel K (2000) The TEA/ATTS transcription factor CaTec1p regulates hyphal development and virulence in *Candida albicans*. *Molecular microbiology* 38 (3):435-445. doi:mmi2132 (pii).
- Chen X, Zhang R, Takada A, Iwatani S, Oka C, Kitamoto T, Kajiwarra S (2017) The role of Bgl2p in the transition to filamentous cells during biofilm formation by *Candida albicans*. *Mycoses* 60 (2):96-103.
- Sundstrom P (2002) Adhesion in *Candida* spp. *Cell Microbiol* 4 (8):461-469. doi:206 (pii).
- Nobile CJ, Mitchell AP (2005) Regulation of cell-surface genes and biofilm formation by the *C. albicans* transcription factor Bcr1p. *Curr Biol* 15 (12):1150-1155. doi:S0960-9822(05)00562-2 (pii) 10.1016/j.cub.2005.05.047.
- Bauer J, Kinast S, Burger-Kentischer A, Finkelmeier D, Kleymann G, Abu Rayyan W, Schröppel K, Singh A, Jung G, Rupp S, and Eickhoff H. (2011) HTS-Identification and SAR-characterization defined (S)-2-(1-aminoisobutyl)-1-(3-chlorobenzyl) benzimidazole as highly antimycotic non-toxic agent. *Journal of Medicinal Chemistry*.
- Kohler T, Wesche S, Taheri N, Braus GH, Mosch HU (2002) Dual role of the *Saccharomyces cerevisiae* TEA/ATTS family transcription factor Tec1p in regulation of gene expression and cellular development. *Eukaryotic cell* 1 (5):673-686.
- Van der Felden J, Weisser S, Bruckner S, Lenz P, Mosch HU (2014) The transcription factors Tec1 and Ste12 interact with coregulators Msa1 and Msa2 to activate adhesion and multicellular development. *Molecular and cellular biology* 34 (12):2283-2293. doi:10.1128/MCB.01599-13
- Bruckner S, Kohler T, Braus GH, Heise B, Bolte M, Mosch HU (2004) Differential regulation of Tec1 by Fus3 and Kss1 confers signaling specificity in yeast development. *Current genetics* 46 (6):331-342. doi:10.1007/s00294-004-0545-1.
- Bauer J, Kinast S, Burger-Kentischer A, Finkelmeier D, Kleymann G, Rayyan WA, Schroppel K, Singh A, Jung G, Wiesmuller KH, Rupp S, Eickhoff H (2011) High-throughput-screening-based identification and structure-activity relationship characterization defined (S)-2-(1-aminoisobutyl)-1-(3-chlorobenzyl)benzimidazole as a highly antimycotic agent nontoxic to cell lines. *J Med Chem* 54 (19):6993-6997. doi:10.1021/jm200571e.
- Daniels KJ, Srikantha T, Pujol C, Park YN, Soll DR (2015) Role of

- Tec1 in the development, architecture, and integrity of sexual biofilms of *Candida albicans*. *Eukaryotic cell* 14 (3):228-240. doi:10.1128/EC.00224-14.
- [15] Staib P, Binder A, Kretschmar M, Nichterlein T, Schroppe K, Morschhauser J (2004) Tec1p-independent activation of a hypha-associated *Candida albicans* virulence gene during infection. *Infection and immunity* 72 (4):2386-2389.
- [16] Francois IE, Aerts AM, Cammue BP, Thevissen K (2005) Currently used antimycotics: spectrum, mode of action and resistance occurrence. *Curr Drug Targets* 6 (8):895-907.
- [17] Okoli I, Coleman JJ, Tampakakis E, An WF, Holson E, Wagner F, Conery AL, Larkins-Ford J, Wu G, Stern A, Ausubel FM, Mylonakis E (2009) Identification of antifungal compounds active against *Candida albicans* using an improved high-throughput *Caenorhabditis elegans* assay. *PLoS one* 4 (9):e7025. doi:10.1371/journal.pone.0007025.
- [18] Pukkila-Worley R, Peleg AY, Tampakakis E, Mylonakis E (2009) *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryotic cell* 8 (11):1750-1758. doi:EC.00163-09 (pii) 10.1128/EC.00163-09.
- [19] Doak TG, Witherspoon DJ, Jahn CL, Herrick G (2003) Selection on the genes of *Euplotes crassus* Tec1 and Tec2 transposons: evolutionary appearance of a programmed frameshift in a Tec2 gene encoding a tyrosine family site-specific recombinase. *Eukaryotic cell* 2 (1):95-102.
- [20] Heise B, van der Felden J, Kern S, Malcher M, Bruckner S, Mosch HU (2010) The TEA transcription factor Tec1 confers promoter-specific gene regulation by Ste12-dependent and -independent mechanisms. *Eukaryotic cell* 9 (4):514-531. doi:10.1128/EC.00251-09.
- [21] Hwang JJ, Chambon P, Davidson I (1993) Characterization of the transcription activation function and the DNA binding domain of transcriptional enhancer factor-1. *EMBO J* 12 (6):2337-2348.
- [22] Lane S, Zhou S, Pan T, Dai Q, Liu H (2001) The basic helix-loop-helix transcription factor Cph2 regulates hyphal development in *Candida albicans* partly via TEC1. *Molecular and cellular biology* 21 (19):6418-6428.
- [23] Colombatti A, Bonaldo P, Doliana R (1993) Type A modules: interacting domains found in several non-fibrillar collagens and in other extracellular matrix proteins. *Matrix* 13 (4):297-306.
- [24] Berman J, Sudbery PE (2002) *Candida Albicans*: a molecular revolution built on lessons from budding yeast. *Nature reviews Genetics* 3 (12):918-930. doi:10.1038/nrg948.
- [25] Sheppard DC, Yeaman MR, Welch WH, Phan QT, Fu Y, Ibrahim AS, Filler SG, Zhang M, Waring AJ, Edwards JE, Jr. (2004) Functional and structural diversity in the Als protein family of *Candida albicans*. *J Biol Chem* 279 (29):30480-30489. doi:10.1074/jbc.M401929200 M401929200 (pii).
- [26] Sanglard D, Hube B, Monod M, Odds FC, Gow NA (1997) A triple deletion of the secreted aspartyl proteinase genes SAP4, SAP5, and SAP6 of *Candida albicans* causes attenuated virulence. *Infection and immunity* 65 (9):3539-3546.
- [27] Biswas S, Van Dijk P, Datta A (2007) Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. *Microbiol Mol Biol Rev* 71 (2):348-376. doi:71/2/348 (pii) 10.1128/MMBR.00009-06.
- [28] Gillum AM, Tsay EY, Kirsch DR (1984) Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae* *ura3* and *E. coli* *pyrF* mutations. *Molecular and General Genetics* MGG 198 (1):179-182.
- [29] Sehnal (2008) Struktur und Funktion des TEA/ATTS-Transkriptionsfaktors Tec1p von *Candida albicans*., der Friedrich-Alexander-Universität Erlangen-Nürnberg, Pöhlten, Österreich.