The mating factor response pathway regulates transcription of *TEC1*, a gene involved in pseudohyphal differentiation of *Saccharomyces cerevisiae*

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Abstract The transcription factor Tec1 is involved in pseudohyphal differentiation and agar-invasive growth of *Saccharomyces cerevisiae* cells. The sole element in the *TEC1* promoter that has thus far been shown to control Tec1 function is the filament response element. We find that the *TEC1* promoter also contains several pheromone response element sequences which are likely to be functional: *TEC1* transcription is induced by mating factor, cell cycle regulated and dependent on the Ste4, Ste18 and Ste5 components of the mating factor signal transduction pathway. Using alleles of the transcription factor Ste12 that are defective in DNA binding, transcriptional induction or cooperativity with other transcription factors, we find little correlation between *TEC1* transcript levels and agar-invasive growth.

Key words: Tec1; Ste12; Mating factor; Haploid-invasive growth

1. Introduction

Under certain conditions of nutrient limitation, diploid *Saccharomyces cerevisiae* cells can adopt a pseudohyphal growth form and haploid cells can invade the surface of an agar substrate [1,2]. These types of growth require a specific signal transduction pathway that consists of several protein kinases and transcription factors, along with other components [2–9]. Several genes involved in nutrient sensing or flocculation also appear to be critical for haploid-invasive and/or pseudohyphal growth [10–14]. Among the proteins required for pseudohyphal differentiation and haploid-invasive growth are the transcription factors Tec1 and Ste12 [3,6,7,15]. Tec1 is a member of the ATTS/TEA group of transcription factors, which are usually involved in developmental processes [6,15]. Ste12 was first identified as a component of the mating factor signal transduction pathway and binding sites for Ste12 (pheromone response elements, PREs, with the consensus sequence TGAAACA) are found in the promoter of many genes involved in the mating factor response [16]. Ste12 also regulates expression of insertions of the transposon Ty1 [17]. The *TEC1* promoter has been shown to contain a PRE element, along with a Tec1 binding TCS element [15]. Together, the combination of the TCS element and a PRE element (called a filamentous growth response element or FRE) mediates the binding of a Tec1/Ste12 dimeric complex which is thought to promote expression of genes like *FLO11* and *TEC1* and stimulate haploid-invasive and pseudohyphal growth [14,15,18]. Fragments containing the FRE sequences from the promoters of *TEC1* and Ty1 have been used to drive expression of LacZ [4,15,19] and expression of such constructs is strongly reduced in cells deleted for *STE12* or *TEC1* [4,15]. Because of the general correlation between expression of these constructs with haploid-invasive and pseudohyphal phenotypes, they are used as reporters for the activity of the pseudohyphal or haploid-invasive pathways [4,15]. It remains to be established whether the FRE element in the *TEC1* promoter is the sole element regulating *TEC1* transcription.

In addition to the TCS and PRE containing FRE element, the *TEC1* promoter has several additional PRE consensus sequences [20]. Clustering of such PRE elements is common to genes involved in the mating factor response pathway and typically confers inducibility by mating factor- [16] and cell cycle-regulated transcription, with high levels in G1 phase and reduced levels as cells enter S phase [21,22]. It is not known whether the PRE consensus sequences in the *TEC1* promoter contribute to its transcriptional regulation. We therefore tested the inducibility of *TEC1* transcription by mating factor, its dependence on components of the mating factor response pathway and its cell cycle regulation.

2. Materials and methods

2.1. Yeast strains and plasmids

The genotypes of the strains used in this study are given in Table 1. Strains were constructed by standard techniques for crossing and gene replacement [23]. Plasmids that provided fragments for the creation of disruption alleles were: pAB506 (*ste2::LEU2* [24]), pM59p7

Table 1
Strains

Strain	Genotype
1255-5C	*MATa bar1*
BOY575	*MATa bar1 ste2::LEU2*
BOY1151	*MATa bar1 ste18::LEU2*
BOY527	*MATa bar1 ste4::LEU2*
BOY1149	*MATa bar1 ste5::LEU2*
BOY1289	*MATa bar1 ste11::TRP1*
BOY763	*MATa bar1 ste7::LEU2*
BOY515	*MATa bar1 kss1::URA3*
BOY517	*MATa bar1 fus3::LEU2*
BOY906	*MATa bar1 kss1::LEU2 fus3::TRP1*
BOY529	*MATa bar1 ste12::LEU2*
BOY501[a]	*MATa bar1 cdc15-2*
L5976[b]	*MATa/MATα*
10560-4D[b]	*MATa*
10560-5B[b]	*MATα*
BOY1480[b]	*MATa ste12::LEU2*

Most strains were isogenic to BF264-15D (*trp1-1a leu2-3,112 ura3 ade1 his2*).
[a]Strain is congenic to W303.
[b]Strains are congenic to Σ1278b [3].

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(*ste18::URA3* [25]), p4-121 (*ste4::LEU2*; V. MacKay, Seattle, WA), pSF32 (*ste5::URA3*; V. MacKay, Seattle, WA), pSL1094 (*ste11::URA3* [26]), pNC113 (*ste7::LEU2* [27]), pBC65 (*kss1::URA3* [28]), pYEE98 (*fus3::LEU2* [29]), pSUL16 (*ste12::LEU2* [30]). Gene disruptions were made by one-step gene replacement with appropriately digested DNA. In some cases the original auxotrophic markers on disruption cassettes were altered using 'marker swap' plasmids [31]. A series of plasmids with wild-type and mutant *STE12* alleles was kindly provided by S. Fields and has been described in detail [32].

2.2. Growth conditions and synchronization procedures

Cells were grown in YEP medium or synthetic dropout medium with dextrose as a carbon source [21]. Agar-invasive growth was assayed essentially as described [2]: patches of cells were first grown for 2 days on selective medium and then replica-plated onto YEPD. After 4 days, photographs were taken to document total growth. To document agar-invasive growth, photographs were taken again after mild rinsing of the plates with water.

Cell cycle synchronization of strains with the thermosensitive *cdc15-2* allele was as described [21]. Cell cycle progression was followed by analysis of transcripts with known patterns of cell cycle regulation [22].

2.3. Northern analysis

Procedures for Northern mRNA analysis were as described previously [33]. Northern blots used in Fig. 2 have been used previously and were reprobed with different probe fragments. *AGA1*, *FUS1* and *CLN2* DNA restriction fragments were excised from low melting point agarose gels, and *SST2, TCM1* and *H2A* fragments were generated by polymerase chain reaction (PCR) as described [22]. A 0.66 kb *Pst*I-*Pvu*II fragment from plasmid pGB821 (kindly provided by J. Boeke) was used as a Ty1-specific probe fragment. A probe fragment containing the entire *TEC1* open reading frame was generated by PCR, using genomic DNA as template and 'Genepair' oligonucleotides against YBR083W (Research Genetics, Huntsville, AL) as primers. The identity of this fragment was confirmed by restriction digest analysis. DNA fragments were radio-labeled by random-prime labeling using a Prime-It kit (Stratagene), and transcript levels were visualized by exposure to autoradiographic film and quantitated using a Molecular Dynamics STORM PhosphorImager system.

3. Results

3.1. TEC1 transcription is induced by mating factor

In addition to the FRE element, the *TEC1* promoter contains several sequences that match six or seven out of seven residues of the PRE consensus sequence TGAAACA (Fig. 1A). Since expression of many genes that contain multiple PRE elements is induced by mating factor, we tested whether *TEC1* transcription is also induced by mating factor. While it has previously been shown that expression of FRE-reporter constructs is not affected by mating factor [15], native *TEC1* transcripts expressed from the full *TEC1* promoter are significantly induced by mating factor (Fig. 1C). This induction is fast (within minutes), just like induction of *FUS1* [34,35] and *SST2* (Fig. 1B), two commonly used reporter genes for activity of the mating factor signal transduction pathway. The speed of induction suggests that the induction is specific and not some indirect effect of cell cycle arrest. In addition, the dose dependence for induction of *TEC1* transcription by mating factor is similar to that of *FUS1* (data not shown). It is therefore likely that the additional PRE elements in the *TEC1* promoter contribute to in vivo transcriptional regulation of *TEC1*.

3.2. TEC1 transcription is cell cycle regulated

We have previously shown that Ste12 is involved in cell cycle regulation of transcription of many genes involved in mating [22]. This regulation likely involves the Ste12 binding PRE sequences that are found in the promoters of these genes. Ste12 has been implicated in *TEC1* transcription through binding to the PRE in the FRE element [15]. To test whether transcription of *TEC1* is cell cycle regulated, we looked at transcript levels in synchronized yeast cells. Cells with a thermosensitive *cdc15-2* allele were grown at permissive temperature and then arrested in late M phase by a shift to restrictive temperature. Synchronous cell cycle progression was then initiated by switching the culture back to permissive temperature. *TEC1* transcript levels were found to fluctuate during the cell cycle: levels were highest in G1 phase and decreased as cells entered S phase (Fig. 2A). A similar pattern was previously found for many genes involved in mating, including *AGA1* (Fig. 2A). In several different synchronization experiments, there was about a five-fold difference in transcript levels between the highest levels in G1 phase and the lowest levels later in the cycle. Other methods of synchronization (elutriation and conditional *CLN* expression [21,22]) gave essentially the s'ame pattern and magnitude of cell cycle-regulated transcription (data not shown). The similarity of the cell cycle-regulated transcription of *TEC1* to that of PRE-con-

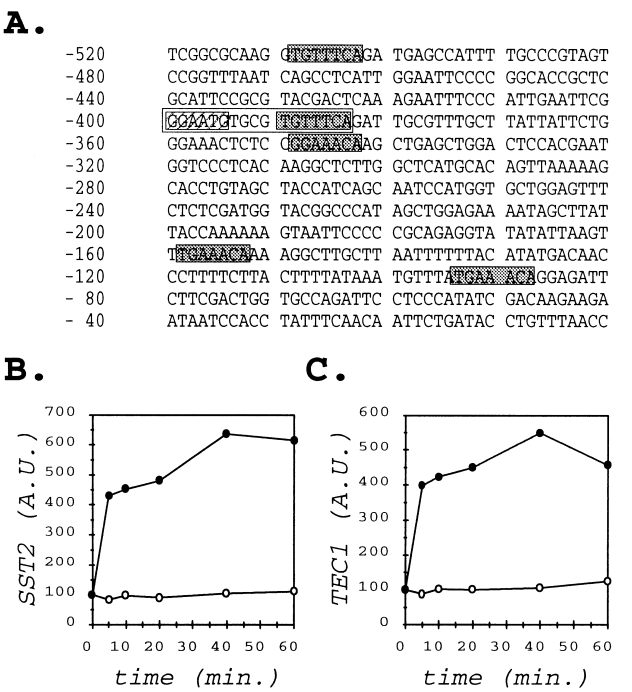

Fig. 1. *TEC1* transcription is induced by mating factor. A: The *TEC1* promoter contains a FRE element and several PRE elements. PRE consensus sequences with six or seven out of seven residues matching the TGAAACA consensus are indicated by a gray box. The TCS sequence GGAATG is indicated by a hatched box. The combined PRE and TCS sequence, known as FRE element, is indicated by a clear box. Position 0 = AUG start codon. B–D: *MATa bar1* wild-type cells (1255-5C) were grown to early exponential phase in YEPD medium and a portion of the culture was then treated with α-mating factor. B, C: Samples were taken for Northern analysis at the indicated times from the treated and untreated cultures. Northern blots were probed for *SST2* (B) and *TEC1* (C) and transcripts were quantitated and corrected for loading in each lane using a PhosphorImager system and *TCM1* transcripts as a loading control. Transcript levels at the time of addition of mating factor were arbitrarily chosen as 100 arbitrary units (AU) and other levels are expressed in relation to this value. Closed symbols represent mating factor treated cells, and open symbols represent untreated controls.

trolled genes [22] is consistent with the idea that the PRE elements in the *TEC1* promoter contribute to its transcriptional regulation.

3.3. Regulation of TEC1 transcription by the mating locus

The expression of many genes involved in mating is restricted to haploid cells [16]. FRE-mediated expression of reporter constructs is significantly (about 13-fold) reduced in diploids compared to haploid cells [15]. We therefore tested whether native *TEC1* expression from its endogenous promoter is affected by the mating locus by looking at transcript levels in MATa/MATα diploid and MATa or MATα haploid cells. *TEC1* transcription was found to be about five-fold reduced in diploid cells when compared to haploid cells (Fig. 2B). This effect was less strong than that of deletion of *STE12* (Fig. 2B), and was much less than the reduction of *SST2* transcription in diploids (Fig. 2B). Although there is a significant reduction in diploid cells, *TEC1* transcription does not appear to be completely restricted to haploid cells.

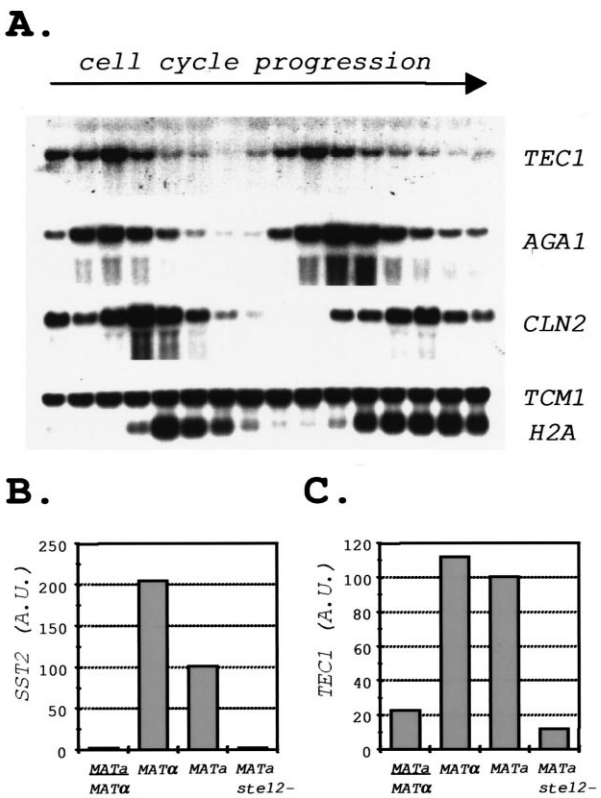

Fig. 2. *TEC1* transcription is cell cycle regulated. A: *MATa cdc15-2* (BOY501) cells were grown to early exponential phase at 25°C and then arrested in late M phase by a shift to 36°C for 3 h. Synchronous cell cycle progression was then started by lowering the temperature back to 25°C. Samples for Northern analysis were taken at the time of the shift to permissive temperature and every 12 min thereafter. B, C: Regulation of *TEC1* transcription by the mating locus. *MATa/MATα* diploids (L5976), *MATa* (10560-4D) and *MATα* (10560-5B) haploids and *MATa ste12::LEU2* (BOY1480) cells were grown to early exponential phase and samples were prepared for Northern analysis. Blots were probed for *SST2* (B) and *TEC1* (C) and transcripts were quantitated and corrected for loading as in Fig. 1B,C. Transcript levels in *MATa* haploids were arbitrarily chosen as 100 arbitrary units (AU), and other levels are expressed in relation to this value.

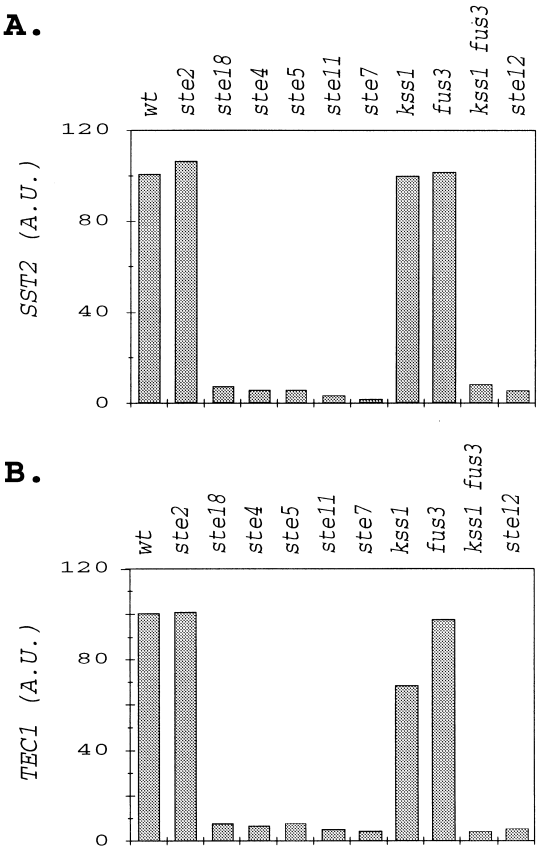

Fig. 3. Dependence of *TEC1* transcription on components of the mating factor signal transduction pathway. Strains with deletions in various components of the mating factor response pathway were grown to early exponential phase and samples were taken for Northern analysis. Blots were probed for *SST2* (A) and *TEC1* (B) and transcripts were quantitated and corrected for loading as in Fig. 1B,C. Transcript levels in wild-type cells were arbitrarily chosen as 100 arbitrary units (AU), and other levels are expressed in relation to this value. Strains used were: 1255-5C (wild-type), BOY575 (*ste2::LEU2*), BOY1151 (*ste18::LEU2*), BOY527 (*ste4::LEU2*), BOY1149 (*ste5::LEU2*), BOY1289 (*ste11::TRP1*), BOY763 (*ste7::LEU2*), BOY515 (*kss1::URA3*), BOY517 (*fus3::LEU2*), BOY906 (*fus3::TRP1 kss1::LEU2*), BOY529 (*ste12::LEU2*).

3.4. Dependence of TEC1 transcription on components of the mating factor response pathway

From previously published observations [15] and the experiments described above, it appears that there are at least two elements that can regulate *TEC1* transcription: (a) the TCS and PRE containing FRE element [15] and (b) other PREs in the *TEC1* promoter indicated in Fig. 1A. To analyze which of these mechanisms contributes most to support basal transcription of *TEC1*, we determined *TEC1* transcript levels in a series of strains with deletions in different components of the mating factor response pathway. The rationale for this experiment is as follows. Components of the mating factor signal transduction pathway include a seven-transmembrane domain receptor that binds mating factor (the *STE2* gene encodes the α-mating factor receptor of MATa cells), a heterotrimeric G-protein (the α, β and γ subunits are encoded by *GPA1*, *STE4* and *STE18* respectively), Ste20 (a protein kinase with homology to p21-activated kinases, PAKs), Ste11 (a MEK kinase homolog), Ste7 (a MEK homolog), Kss1/Fus3 (two MAP kinase homologs), Ste5 (a protein which is though to function as a

molecular scaffold to enhance the function of several protein kinases in the pathway) and the transcription factor Ste12 (for a review of this pathway and for references see [16]). It has previously been shown that FRE-mediated expression of reporter constructs strongly depends on Ste11, Ste7 and Ste12, while it does not depend on Ste4 and Ste5 [15]. If the FRE element is the main transcriptional element, *TEC1* transcription would also be expected to depend on Ste12, Ste11 and Ste7 and not on Ste4 and Ste5. In contrast to FRE-controlled transcription, transcription of PRE-controlled genes (e.g. *FUS1* and *SST2*) is strongly dependent on both the Ste11, Ste7, Ste12 group of proteins and the Ste4, Ste18 and Ste5 group (e.g. [26]). Therefore, if the PRE elements are the main elements supporting basal transcription of *TEC1*, a strong dependence of *TEC1* transcription on Ste4, Ste18 and Ste5 is anticipated. As expected from a PRE-controlled gene, the transcription of *SST2* was found to be sensitive to the function of Ste4, Ste18, Ste5 along with Ste11, Ste7 and Ste12 (Fig. 3A). *TEC1* transcription was also found to equally depend on Ste4, Ste18 and Ste5 as on Ste11, Ste7 and Ste12 (Fig. 3B). This suggests that, at least under these conditions, PRE elements are the main elements supporting basal transcription of *TEC1*.

3.5. Effects of Ste12 mutation on TEC1 and Ty1 transcription

Ste12 is involved in both the basal transcription of many genes involved in mating and in their transcriptional induction by mating factor. Besides its interaction with Tec1, Ste12 can interact with another transcription factor, Mcm1, to promote the transcription of *STE2* [36] and *FAR1* [22], a gene required for cell cycle arrest in response to mating factor. Domains in Ste12 have been identified that are required for DNA binding, cooperation with Mcm1 and for transcriptional induction by mating factor. Kirkman-Correia and Fields have generated Ste12 mutants that are defective in specific aspects of Ste12 function [32]. Mutants which are defective in DNA binding (*STE12Δ104–219* and *STE12Δ172–252*) and mutants which are thought to be defective for transcriptional activation (*STE12Δ383–669* and *STE12Δ305–587*) do not support basal and induced *FUS1* transcription and do not complement the mating defect of *ste12*$^-$ cells [32]. Mutants that support basal transcription of *FUS1* but do not allow for transcriptional induction by mating factor (*STE12Δ253–335* and *STE12Δ255–354*) also do not complement the mating defect of *ste12*$^-$ cells [32]. Mutants that lack the interaction domain for Mcm1 (*STE12Δ436–669* and *STE12T435*) are proficient in all mating functions [32]. We tested whether the regulation of *TEC1* transcription in cells with plasmids containing wild-type *STE12* or these various classes of *STE12* mutants. *TEC1* transcription appeared to be lowest in cells with Ste12 mutants which were low in *SST2* transcription, and highest in cells with Ste12 mutants that allow for high *SST2* transcript levels (Fig. 4B,C). The differences in *TEC1* transcription in the various Ste12 mutants were not as large as for *SST2*, but in general a similar pattern was observed (Fig. 4B,C). The pattern of transcription of *TEC1* transcription in the various mutants was also similar to that of Ty1 (Fig. 4D). These findings show another situation where *TEC1* transcription is similar to that of PRE-controlled genes like *SST2*. This supports the general idea that the PRE-elements in the *TEC1* promoter are important for its transcriptional regulation.

3.6. TEC1 and Ty1 transcription do not correlate with haploid-invasive growth

In the strains with various Ste12 mutants we then tested

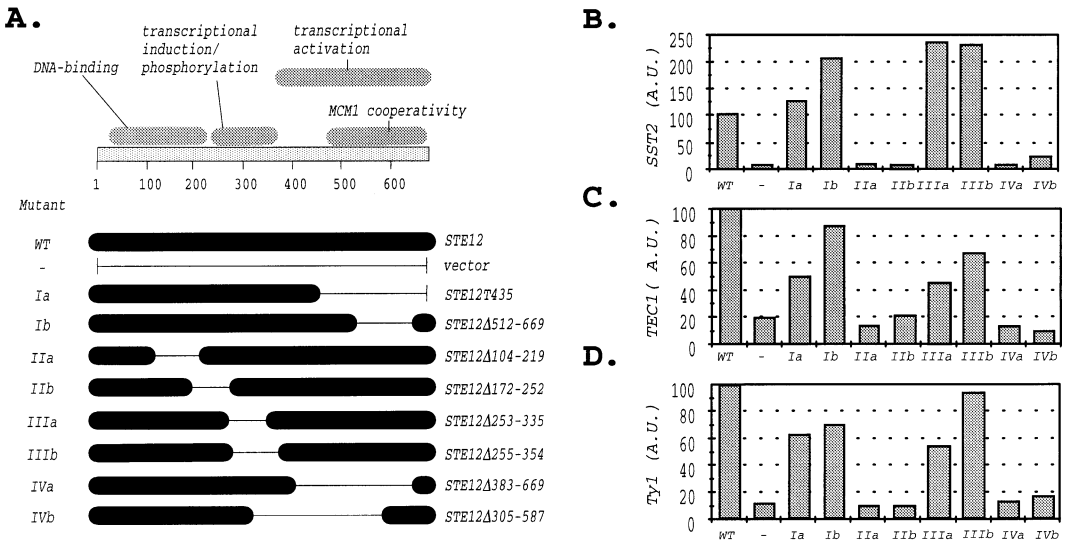

Fig. 4. *TEC1* transcription in strains with mutant *STE12* alleles. A: Graphic summary of the different domains of Ste12, as identified by Kirkman-Correia and Fields, and of the various Ste12 mutants used [32]. Mutants are divided into four classes with two representatives each: class I mutants are defective for interaction with Mcm1, (Ia = *STE12Δ436–669* and Ib = *STE12T435*); class II mutants have deletions in the DNA binding domain (IIa=*STE12Δ104–219* and IIb=*STE12Δ172–252*); class III mutants are defective in transcriptional induction by mating factor (IIIa = *STE12Δ253–335* and IIIb = *STE12Δ255–354*); class IV mutants are defective for transcriptional activation (IVa = *STE12Δ383–669* and IVb = *STE12Δ305–587*). Black bars indicate the expressed parts of the protein. B–D: Transcript levels in *MATa ste12::LEU2* cells (BOY1480) carrying vectors with the wild-type *STE12* gene or mutant *STE12* alleles indicated in A. Cells were grown on SCD-ura medium to exponential phase and samples were prepared for Northern analysis. Blots were probed for *SST2* (B), *TEC1* (C) and Ty1 (D) and transcripts were quantitated and corrected for loading as in Fig. 1B,C. Lanes are marked by Roman numerals which refer to the mutant genotypes indicated in A. Transcript levels in cells with plasmids containing the wild-type *STE12* gene were arbitrarily chosen as 100 arbitrary units (AU), and other levels are expressed in relation to this value.

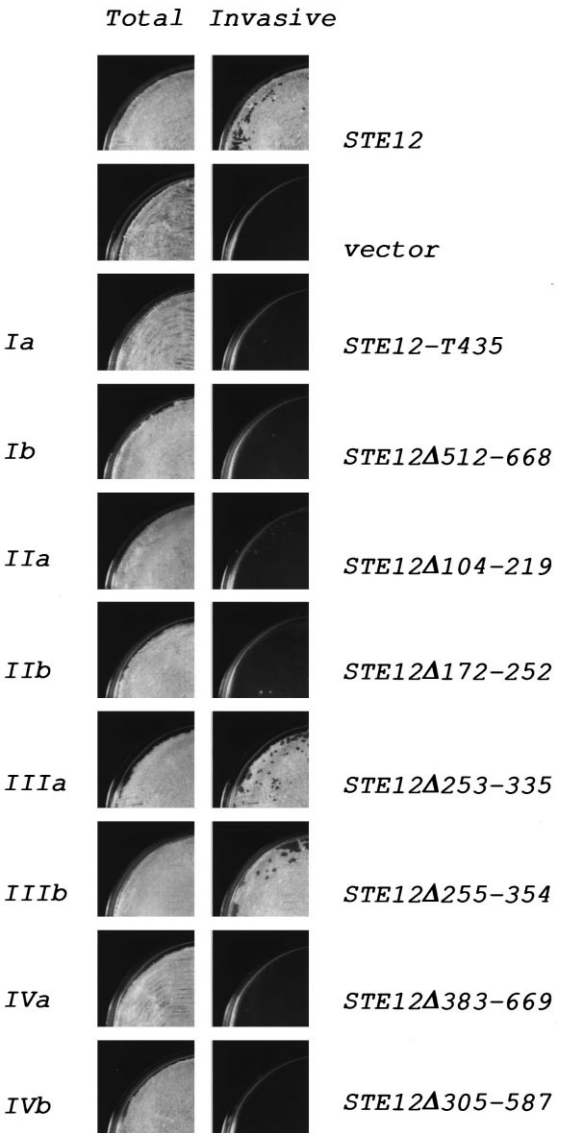

Fig. 5. Agar-invasive growth with different *STE12* mutants. Strains with the different *STE12* alleles indicated in Fig. 4A were tested for haploid-invasive growth as described in Section 2. The same strains were used as for Fig. 4.

whether they were proficient in haploid-invasive growth. It appears that, besides the wild-type positive controls, only the mutants that are defective in mediating transcriptional induction by mating factor (*STE12Δ253–335* and *STE12Δ255–354*) can support haploid-invasive growth, while other mutants that show similar levels of basal *TEC1* transcription (*STE12Δ436–669* and *STE12T435*) are defective in haploid-invasive growth (Fig. 5). Because the latter mutants (*STE12Δ436–669* and *STE12T435*) are proficient in mating functions [32] but defective in haploid-invasive growth and the former mutants (*STE12Δ253–335* and *STE12Δ255–354*) are proficient in haploid-invasive growth while defective in mating functions, these results indicate that the requirement of Ste12 function in the haploid-invasive pathway is different from that in the mating factor signal transduction pathway.

4. Discussion

4.1. Transcriptional regulation of TEC1

In many respects, *TEC1* transcripts are regulated like *AGA1*, *SST2* or *FUS1*, which are used to monitor the activity of the mating factor response pathway. Like *SST2* and *FUS1*, *TEC1* transcription is induced by mating factor, it shows a similar dependence on components of the mating factor response pathway and a similar pattern of cell cycle regulated transcription. Since many genes that show such a pattern of expression are involved in the mating process, this raises the question whether Tec1 has some previously unidentified role in mating. It has been shown that cells deleted for *TEC1* are proficient in mating and support MFα1 transcription [20], indicating that Tec1 is not involved in haploid-specific gene expression or mating. In agreement with this, we found in quantitative assays that the mating efficiency of *tec1*$^-$ cells to tester strains in liquid culture or on filters was similar to that of wild-type cells (data not shown). The increase in native *TEC1* transcription by mating factor does not seem to have any consequences for FRE-mediated reporter gene transcription [15]. It could be that Tec1 has a more subtle role in the mating reaction, which has yet to be discovered.

Mutants in TCS or PRE sequences of the FRE have been generated in the context of the full *TEC1* promoter [15]. Either mutation leads to marked defects in pseudohyphal development [15], indicating that the FRE element contributes significantly to regulation of the function of *TEC1*. However, certain constructs in which the *TEC1* TCS sequence was missing could complement effects of *TEC1* deletion on Ty1-mediated transcription [20]. Apparently a 394 bp fragment of the *TEC1* promoter that lacks the TCS sequence, can support sufficient *TEC1* transcription to allow for complementation of certain phenotypes of *tec1*$^-$ cells [20]. This indicates that binding of Tec1 to elements in its own promoter is not essential for some functions of Tec1, but that it is for other functions, like pseudohyphal growth.

4.2. TEC1 transcription as a reporter for haploid-invasive growth

It appears that regulation of *TEC1* transcription driven from its whole promoter is quite different from the regulation of FRE-supported transcription. FRE-mediated transcription is not induced by mating factor and does not depend on factors like Ste4, Ste5 and Ste18 [15] while native *TEC1* transcription does. There seems to be a general correlation between FRE-mediated transcription and haploid-invasive or pseudohyphal growth, but there appears to be little correlation between native *TEC1* transcription and haploid-invasive growth. The clearest examples at the opposite ends are *ste4*$^-$ cells (which show strongly reduced *TEC1* transcript levels, while haploid-invasive growth is normal [2]) and *STE12Δ436–669* or *STE12T435* mutants (which have fairly normal *TEC1* transcript levels, but are deficient in haploid-invasive growth). Since Tec1 is required for haploid-invasive growth, the observations in *ste4*$^-$ cells also raise the question how *ste4*$^-$ cells, which have such markedly reduced *TEC1* transcript levels, can be normal for haploid-invasive growth. A similar question holds for diploid cells, which have significantly reduced transcript levels of *TEC1*, although in this case the effect is not as strong as elimination of Ste12. Apparently the remaining *TEC1* transcript level in diploid and *ste4*$^-$ cells

is sufficient to allow for pseudohyphal or haploid-invasive growth. It is possible that the specific conditions that induce pseudohyphal or haploid-invasive growth will stimulate *TEC1* expression. However, in preliminary experiments we found no evidence for induction of *TEC1* transcription in diploids in low ammonium liquid media (data not shown), as was recently observed for *FLO11* [14]. On the whole, there seems to be little correlation between native *TEC1* transcript levels and the haploid-invasive phenotype.

4.3. Regulation of haploid-invasive and pseudohyphal growth

It should be noted that the *STE12Δ436–669* and *STE12T435* mutants, which are defective in Mcm1 cooperativity [32], are proficient in supporting *TEC1* transcription. This indicates that cooperativity of Ste12 with other transcription factors through the C-terminal domain is not required to support native *TEC1* transcription. However, this domain appears to be critical for supporting haploid-invasive growth, suggesting that the interaction of Ste12 with another transcription factor is required for this. Tec1 and Ste12 have previously been shown to bind cooperatively to FRE sequences in the *TEC1* promoter [15]. These findings suggest the possibility that the interaction of Ste12 with Tec1 occurs through the same domain that is required for integration of Ste12 with Mcm1.

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