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MINI-REVIEW

Regulation of transcription of cellulases- and hemicellulases-encoding genes in *Aspergillus niger* and *Hypocrea jecorina (Trichoderma reesei)*

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Abstract The filamentous fungi *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*) have been the subject of many studies investigating the mechanism of transcriptional regulation of hemicellulase- and cellulase-encoding genes. The transcriptional regulator XlnR that was initially identified in *A. niger* as the transcriptional regulator of xylanase-encoding genes controls the transcription of about 20–30 genes encoding hemicellulases and cellulases. The orthologous *xyr*1 (xylanase regulator 1-encoding) gene product of *H. jecorina* has a similar function as XlnR, although at points, the mechanisms seems to be different. Specifically in *H. jecorina*, the interaction of Xyr1 and the co-regulators Ace1 and Ace2 in the regulation of transcription of xylanases and cellulases has been studied. This paper describes the similarities and differences in the transcriptional regulation of expression of hemicellulases and cellulases in *A. niger* and *H. jecorina*.

Keywords Transcriptional regulation · Gene expression · Xylanase · Cellulase

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Introduction

The vast amount of plant biomass waste produced by the agro-technological industry forms an attractive potential as a renewable source for energy and basic chemicals (Hahn-Hägerdal et al. 2006; Himmel et al. 2007; Ragauskas et al. 2006). One of the bottlenecks in fully exploiting this potential is the efficient hydrolysis of the cellulose and hemicellulose fractions to its monomeric compounds. These monomeric constituents are mainly sugars as D-glucose and D-xylose.

Many microorganisms have the capacity to degrade plant biomass. Filamentous fungi like *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*) have been shown to produce a wide spectrum of polysaccharide-hydrolytic enzymes. Of these fungi, *A. niger* is industrially used as a producer of many pectinases (Bussink et al. 1992; Delgado et al. 1992; Parenicová et al. 2000) and hemicellulose-degrading enzymes, like xylanases and arabinases (Gielkens et al. 1997; van Peij et al. 1997), while *H. jecorina* is the most important organism used in cellulase production (Fägerstam and Pettersson 1980; Irwin et al. 1993; Nidetzky et al. 1994; Wood and McCrae 1979).

From the start of the research on cellulose degradation, a number of intriguing biological questions have been posed (Kubicek et al. 1993): How is the fungus able to sense the insoluble polymeric substrate? What are the signalling molecules? How is the system regulated, and does D-glucose act as a repressor of the system?

While some of these questions can be answered nowadays, a number is still open today. Furthermore, new questions arise, as the outcome of the research suggests that the mechanisms, working in the various organisms studied, show clear similarities but also significant differences in the regulation

of the transcription of cellulase- and hemicellulase-encoding genes. Because in filamentous fungi, the regulatory mechanisms of cellulase and hemicellulase expression are by far best studied in *A. niger* and *H. jecorina*, in this paper, we give an overview of the transcription factors involved and their mode of (inter)action in these fungi. Remarkably, the regulation of the transcription of the cellulolytic and hemicellulolytic enzyme-encoding genes occurring in both organisms has many similarities but shows also some important differences.

XlnR is a transcriptional activator of xylanase and cellulase expression in *A. niger*

A promoter deletion analysis of the *Aspergillus tubingensis* regulatory *xlnA* gene in *A. niger* was the basis for the later cloning of the *xlnR* gene. As a result of this analysis, a fragment was identified that contained an element that was able to induce the transcription of the downstream gene (de Graaff et al. 1994). By using this fragment in front of the *pyrA* gene of *A. niger*, which was then controlled by XlnR such as the *xlnA* gene, xylanolytic regulatory mutants were isolated. A transformant harbouring this construct was used to develop a genetic screen, allowing the isolation of regulatory mutants, which lacked xylan- or D-xylose-induced expression of the *xlnR* gene on the basis of 5-fluoroorotic acid resistance. Ten mutants that lacked xylanase and β-xylosidase expression were isolated, and the complementation of one of these resulted in the cloning of the *xlnR* gene (van Peij et al. 1998b). This was the first cloning of a transcription factor involved in xylanase and cellulase expression in a filamentous fungus.

The *xlnR* gene of *A. niger* encodes a binuclear zinc finger protein, which is a class of transcription factors specific for fungi to which, e.g. also *Saccharomyces cerevisiae* GAL4p belongs. The protein encoded is 875 amino acids in length and shows a marked binuclear zinc finger deoxyribonucleic acid (DNA)-binding domain. This DNA-binding domain binds to the sequence 5′-GGCTAA-3′ that is present in triplicate within the *xlnA* promoter fragment, as was determined by DNaseI foot printing (de Graaff et al. 1994).

The role of XlnR as a transcriptional activator is not restricted to genes encoding enzymes of the xylanolytic system of *A. niger*. By Northern blot analysis, XlnR was shown to direct also the transcription of a number of endo-cellulases and two cellobiohydralases but not that of β-glucosidase. The XlnR-binding motif is found in the upstream regulatory regions of all these genes in *A. niger* that are controlled by XlnR, except for the gene encoding cellobiohydrolase B (Gielkens et al. 1999). Furthermore, some of the galactosidases-encoded genes of *A. niger* were shown to be under control of XlnR (de Vries et al. 1999a).

It was Hasper et al. (2000) who showed the interconnection of extracellular xylan degradation and intracellular D-xylose metabolism is coupled via the transcriptional regulation of the D-xylose reductase-encoding *xyrA* gene by XlnR. In this manner, the fungus is able to adapt intracellular D-xylose metabolism to extracellular xylan degradation. Recently, the genome sequence of *A. niger* has become publicly available. The *A. niger* genome contains about 14,600 genes, of which about 170 are involved in polysaccharide degradation (Pel et al. 2007). Based on this genome sequence, micro-array analysis of an *A. niger xlnR* wild type and *xlnR* knockout strain has been performed (Trindade, van der Veen and de Graaff, manuscript in preparation). The preliminary results of this analysis show about 25 genes being positively regulated by XlnR. The enzymes encoded are mostly involved in the modification and hydrolysis of arabinoxylans and glucans. In addition, three sugar transporters were identified of which the transcription is found to be controlled by XlnR, which is in accordance with the findings of Hasper et al. (2000). They showed that decreased D-xylose uptake is a result of promoter titration in strains having a high number of copies of a XlnR controlled gene. An overview of the genes of the XlnR regulon, as was determined both by Northern blot analysis and by micro-array analysis, including their known or putative function, is given in Table 1.

The above-described results have shown the broader role for XlnR. It appears to be an important transcriptional regulator in the expression of polysaccharide-degrading enzymes and the connecting intracellular carbon metabolism in *A. niger*. On the basis of these findings, the enzyme cascade model for the induction of polysaccharide-degrading enzyme expression was proposed (Gielkens 1999; Fig. 1). Essentially, this model assumes that carbon limitation leads to a carbon catabolite-de-repressed condition, and in this state, monomeric sugars or the their derivates can act as inducers. The nature of the monomeric sugar determines the polysaccharide enzyme system to be induced. In Fig. 1, D-xylose is given as an example of a monomeric inducer that activates the XlnR regulon.

Functional domains in *A. niger* XlnR

The XlnR protein belongs to the class of zinc binuclear cluster domain proteins (PF00172) like, e.g. GAL4 (Finn et al. 2006). The bi-nuclear cluster domain forms the DNA-binding domain and is found in XlnR at the N-terminal part of the protein, formed by amino acids from position 49 till 91. In this region, only two amino acid changes are found compared to the DNA-binding domain of the XlnR orthologue in *H. jecorina*, Xyr1 (xylanase regulator 1; Stricker et al. 2006; Rauscher et al. 2006).

Table 1 Abbreviations in alphabetical order[a]

Abbreviation	Meaning
Ace1	Activator of cellulases 1, *H. jecorina*
Ace2	Activator of cellulases 2, *H. jecorina*
Aip	Ace1-interacting protein, *H. jecorina*
BGL I/II	β-Glucosidase I/II, *H. jecorina*
BXL I	β-Xylosidase I, *H. jecorina*
C2,… C8	Glucose oligomers resulting from cellulose degradation
CBH I/II	Cellobiohydrolase I/II, *H. jecorina*
EGL I	Endoglucanase I, *H. jecorina*
EMSA	Electrophoretic mobility shift assay
Glc/G	Glucose
GAL4	Encodes a positive regulator of galactose -inducible genes in *S. cerevisiae*
GFP	Green fluorescent protein
SO	Sophorose
UAS	Upstream activating sequence
X1	Xylose
X2,… X8	Xylose oligomers resulting from xylan degradation
XAE	Xylanase-activating element
XlnR	Xylanase regulator, *A. niger*
XYN I/II	Xylanase I/II, *H. jecorina*
Xyr1	Xylanase regulator 1, *H. jecorina*

[a] Except gene names mentioned in Table 2

In addition to the DNA-binding domain at positions 481 to 576, a fungal specific transcription factor domain is found (PF04082). This domain is found in many transcription factors of PF00172 that are involved in metabolic processes, but the function of this domain remains thus far unknown (MacPherson et al. 2006).

The mutant collection of XlnR loss-of-function mutants generated by van Peij was the starting point for a structure function analysis of *A. niger* XlnR. For this functional study, green fluorescent protein (GFP) fusions of mutated XlnR proteins were made. From the work of van Peij et al. (1998b), it had become clear that a putative coiled-coil domain is important for the XlnR function, as the disruption of the α-helix structure by a Leu650Pro mutation leads to a loss of function of XlnR. The GFP fusion studies gave insight in the mechanism behind this, as the deletion of the region that forms an α-helix structure leads to a cytoplasmic localisation of XlnR and, as a result, to a loss of the transcription of the structural genes of the regulon. From this, it was concluded that this structure is involved in the nuclear localisation of the protein (Hasper et al. 2004).

The same study showed that the C-terminal part of the XlnR protein is involved in the transcriptional regulation. A deletion of 78 C-terminal amino acids leads to an increased expression of the XlnR target genes, also under D-glucose repression conditions (Hasper et al. 2004), suggesting this region is a modulation domain in the protein. Very similar mechanisms for the modulation of the transcriptional activity of the *S. cerevisiae* Leu3p regulator have been described (Wang et al. 1997). Single-amino acid mutations in this region lead to a loss of function of XlnR. The findings are summarised in Fig. 2.

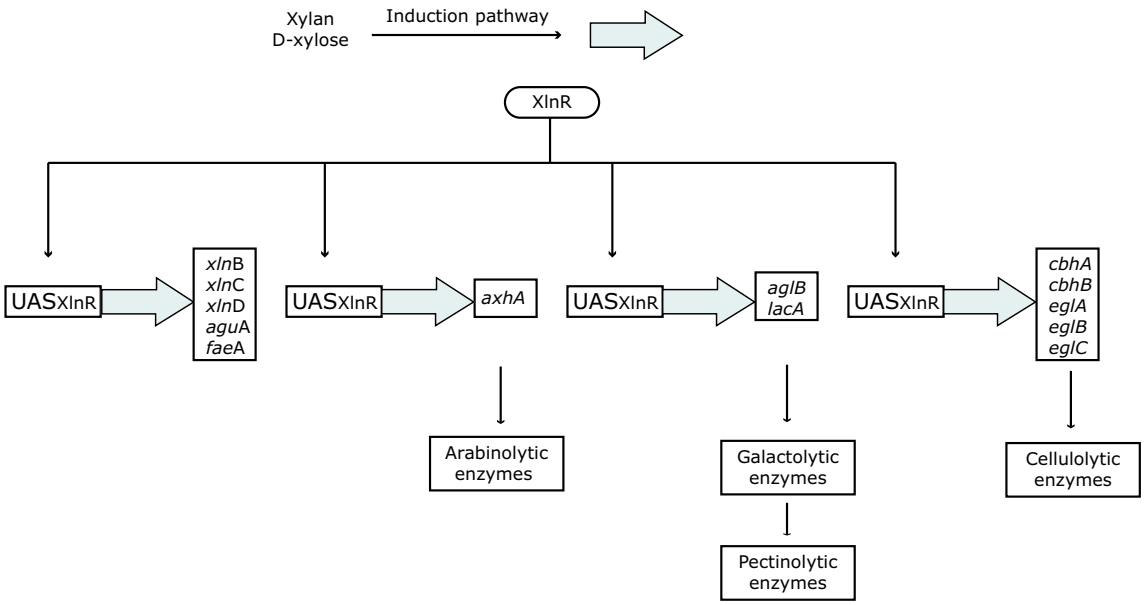

Fig. 1 Schematic model for the regulation of genes encoding enzymes (Table 1) involved in the degradation of plant cell wall polysaccharides in *A. niger*. The model visualizes how the release of sugars different to D-xylose by the action of enzymes, whose expression is controlled by the binding of XlnR to the upstream-activating sequence (*UAS*) in the promoter leads to the induction of enzyme systems not controlled by XlnR. Thus, these sugars and sugar oligomers itself serve as the inducers of the expression of polysaccharidases that are controlled by transcription factors different to XlnR (modified to Gielkens 1999)

Table 2 Genes controlled by *A.niger* XlnR

Gene name	Functional annotation	E.C. number	Reference
Enzymes involved in xylan degradation			
XlnC	Endo-1,4-β-xylanase C	3.2.1.4	(Gielkens et al. 1999)
XlnB	Endo-1,4-β-xylanase B	3.2.1.8	(Kinoshita et al. 1995)
XlnD	β-Xylosidase	3.2.1.-	(van Peij et al. 1997)
AxhA	1,4-β-d-Arabinoxylan arabinofuranohydrolase	3.2.1.55	(Gielkens et al. 1999)
AxeA	Acetyl xylan esterase	3.1.1.73	(de Graaff et al. 1992)
aguA	α-Glucuronidase	3.2.1.139	(de Vries et al. 2002)
estA	Esterase A	3.1.1.3	(Bourne et al. 2004)
faeA	Ferulic acid esterase A	3.1.1.73	(de Vries and Visser 1999)
	Putative xylosidase		[a]
Enzymes involved in cellulose and xyloglucan degradation			
cbhA	1,4-β-d-glucan cellobiohydrolase A	3.2.1.91	(Gielkens et al. 1999)
cbhB	1,4-β-d-glucan cellobiohydrolase B	3.2.1.91	(Gielkens et al. 1999)
eglA	Endo-1,4-β-glucanase A	3.2.1.4	(van Peij et al. 1998a)
eglB	Endo-1,4-β-glucanase B	3.2.1.4	(van Peij et al. 1998a)
eglC	Endo-1,4-β-glucanase C (xyloglucanase)	3.1.1.72	(Hasper et al. 2002)
	Putative cellulases		[a]
	Putative xyloglucanases		[a]
	Enzymes involved in D-xylose metabolism		
xyrA	D-Xylose reductase	1.1.1.200	(Hasper et al. 2000)
talB	TalB trans-aldolase-like	2.2.1.2	[b]
	3 Putative sugar transporters		[a]
Other systems			
aglB	α-Galactosidase B	3.2.1.22	(de Vries et al. 1999a)
lacA	β-Galactosidase lacA	3.2.1.23	(de Vries et al. 1999a)
	Putative transcription factor		[a]

[a] Trindade et al., manuscript in preparation
[b] Hasper and de Graaff, unpublished

Xylanase expression in *H. jecorina*: different inducers activate different xylanolytic genes

Early studies establishing the culture conditions for an accumulation of xylanolytic activity reported abundant production, when *H. jecorina* is cultivated on media containing cellulose, xylan or mixtures of plant polymers (reviewed by Bisaria and Mishra 1989; Kubicek 1993; Zeilinger and Mach 1998). Because these very potent natural-inducing compounds cannot enter the fungal cells, it is obvious that oligosaccharides released from these polymers and/or respective derivatives function as the actual substances triggering the induction of xylanase expression.

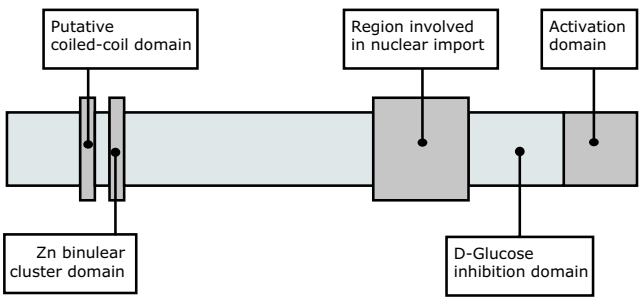

Fig. 2 Schematic representation of the functional domains in *A. niger* XlnR protein (modified to Hasper 2003). The protein is oriented from the N (*left*) to C termini (*right*). The Zn binuclear cluster DNA-binding domain is found at the N terminus of the protein. Based on expression and localisation studies in XlnR mutated proteins, it is assumed that the central coiled-coil domain is involved in the nuclear localisation. The C-terminal region of this domain seems to be involved in the regulation of the XlnR activity and contains a presumed D-glucose inhibition domain and an activation domain. The latter might be involved in intra- or inter-molecular interactions

Whereas in *Aspergillus*, the xylanolytic and cellulolytic system is strictly co-regulated via the inducer D-xylose (e.g. Gielkens et al. 1999; Hasper et al. 2000), enzymes participating in the respective *H. jecorina* hydrolytic complexes are not. Their differential expression has been reported in several studies: Culturing *H. jecorina* on cellulose or xylan causes the formation of two specific endo-β-1,4-xylanases (XYN I and XYN II) and one single unspecific endo-glucanase (EGL I; Senior et al. 1989). Applying sophorose (the best to date known cellulase-inducing compound in *H. jecorina* [two β-1,2-linked D-glucose units]), which is considered as the natural inducer of cellulase formation (e.g. Mandels et al. 1962; Mandels and Reese 1960; Sternberg and Mandels 1979), only one of both xylanases (XYN II) and endoglucanase I can be detected,

whereas xylobiose leads to the formation of both xylanases and a β-xylosidase but not of endoglucanase I (Hrmova et al. 1986; Margolles-Clark et al. 1997). Finally, the *xyn1* transcription, in contrary to all other xylanolytic and cellulolytic enzyme-encoding genes in *H. jecorina*, is induced by D-xylose (Mach et al. 1996). More recently, these data were confirmed by transcriptional analysis. In addition, evidence for a different transcript formation pattern of these two xylanases with respect to D-glucose was provided (Mach et al. 1996; Zeilinger et al. 1996). It was demonstrated that the *xyn2* transcript arises at a low basal level when the fungus is grown on D-glucose as the sole carbon source. This basal level is elevated in the presence of xylan, xylobiose or sophorose. The simultaneous presence of D-glucose and xylan leads to a drop of the transcription to the basal level, whereas the induction of the *xyn2* transcription by xylobiose is not affected by D-glucose (Würleitner et al. 2003). However, the *xyn1* transcription which is induced by D-xylose is not affected by the simultaneous presence of D-glucose and D-xylose, whereas D-glucose alone completely represses its expression (Mach et al. 1996; Rauscher et al. 2006). Recently, a general model for the substrate recognition and the induction mechanisms of the major xylanolytic and cellulolytic enzymes of *H. jecorina* (Stricker et al. 2006) including the general in trans-acting activator Xyr1 (Fig. 3) was postulated.

The model summarises all the discussed hydrolytic enzyme-encoding genes, which are inducible by the respective degradation and/or transglycosylation products of xylan and/or cellulose. While *xyn1* is inducible by the monomer of xylan, D-xylose (Mach et al. 1996), the *xyn2* gene is inducible by the dimer of xylan, xylobiose and the transglycosylation product sophorose (Zeilinger et al. 1996). The gene *bxl1* encodes for a β-xylosidase (which converts xylobiose to D-xylose) and is inducible by xylobiose (Margolles-Clark et al. 1997). The cellulase-encoding genes *cbh1*, *cbh2* and *egl1* (Ilmén et al. 1997) as well as *bgl1* (Fowler and Brown 1992) and *bgl2* (Saloheimo et al. 2002) have been reported to be inducible by sophorose. Furthermore, the model points at the fact that in all cases, Xyr1 (except for *bgl2*) is an indispensable trans-activator (Stricker et al. 2006).

Xyr1 is the general activator of hydrolase formation in *H. jecorina*

In contrast to the diversity of inducers and induction mechanisms (described vide supra), it was recently demonstrated that the transcriptional regulation of the major hydrolytic enzyme-encoding genes *xyn1*, *xyn2* (Xylanases I and II-encoding), *cbh1*, *cbh2* (Cellobiohydrolases I and II-encoding) and *egl1* (endoglucanase I-encoding) is strictly dependent on Xyr1. Regulation of the respective genes via Xyr1 is not affected by the substances mediating the induction (degradation and transglycosylation products, such as D-xylose, xylobiose, sophorose as well as the inducer molecule lactose) and is indispensable for all modes of gene expression (Stricker et al. 2007a; Stricker et al. 2006). Id est, neither the Cre1 (carbon catabolite repressor protein 1)-dependent de-repressed or induced transcriptional level of *xyn1* (Mach et al. 1996) nor the basal or induced transcriptional level of *xyn2* (Zeilinger et al. 1996) could be observed in a Δ*xyr1* strain. The Cre1

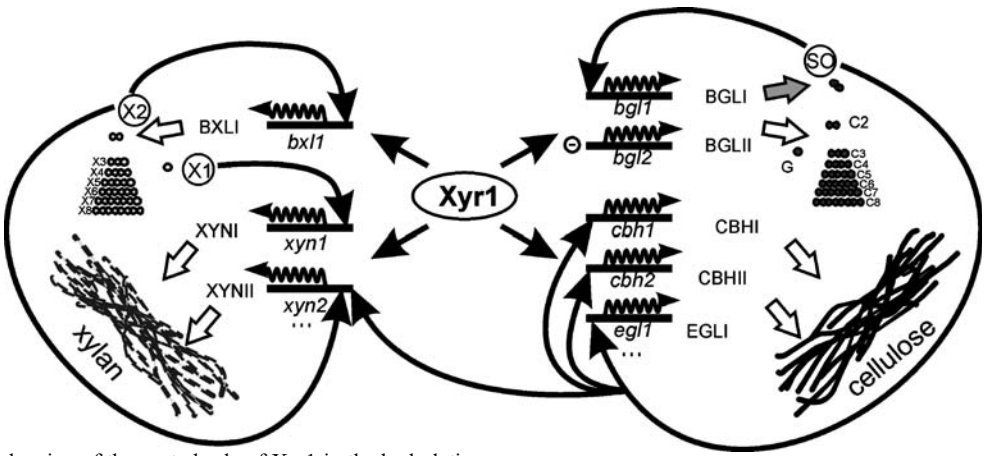

Fig. 3 Schematic drawing of the central role of Xyr1 in the hydrolytic enzyme system of *H. jecorina* causing degradation of xylan and cellulose. Xyr1 acts on both: (1) as an in trans-acting factor directly on the expression of xylanases (as *XYNI*, *XYNII*) and cellulases (as *CBHI*, *CBHII*, *EGLI*) as well as (2) on the corresponding inducer (D-xylose, *X1*)-providing enzyme β-xylosidase I (*BXLI*) in the case of xylan utilization and on the corresponding inducer (sophorose, *SO*)-providing enzyme β-glucosidase I (*BGLI*) in the case of cellulose utilization. *White arrows* indicate enzymatic degradation of the respective biopolymer to oligo- or monomeric sugars (*X1–X8* and *G*, *C2–C8*). *Grey arrow* indicates the transglycosylation activity of BGLI and II. *Minus sign* indicates the Xyr1-independent expression of the *bgl2* gene

(*H. jecorina*)/CreA (*A. niger*) protein has for both organisms been described as a wide domain repressor of particular hydrolase-encoding genes (e.g. de Graaff et al. 1994; de Vries et al. 1999b; Ilmen et al. 1996; Mach et al. 1996). Whereas only some of the major hydrolases in *H. jecorina* namely, *cbh1* and *xyn1*, are under direct Cre1 control (Ilmen et al. 1996; Mach et al. 1996), other hydrolytic enzyme-encoding genes such as *cbh2*, *xyn2* and *bgl1* are not regulated Cre1 dependent (Margolles-Clark 1997; Würleitner et al. 2003; Zeilinger et al. 2003).

Currently, evidence was provided that the corresponding in cis-acting elements (Xyr1-binding sites) occur as inverted repeats in both promoters (*xyn1* and *xyn2*) with a spacing of 10 or 12 bases, respectively. In case of the regulation of the *xyn1* expression, it was recently reported that the induction-specific protein–DNA complex contains two Xyr1 proteins, each contacting one of the inverted repeats (Rauscher et al. 2006). Corresponding inverted elements in the *xyn2* promoter are likewise bound by in vitro translation products of xyr1, whereas a mutation in either one of these motives fully abolishes binding of Xyr1 (Stricker et al. 2007b). This finding strongly suggests that Xyr1 dimers can only contact a repeat of its binding element. As a summary, it can be concluded that Xyr1 seems to dimerise before binding to its motives, but dimerisation is not essential for binding.

As mentioned above, Xyr1 is the essential activator for all levels of *xyn1* and *xyn2* transcription, obviously directly or indirectly receiving and mediating all different signals from inducer molecules. During recent years, not only evidence for the involvement of additional wide domain regulators (e.g. Hap2/3/5 [CCAAT-binding protein] and Cre1; Mach et al. 1996; Rauscher et al. 2006; Würleitner et al. 2003; Zeilinger et al. 1996) could be demonstrated but recently also strong indications for the participation of particularly specific transcription factors, notably Ace1 (activator of cellulases 1) and Ace2 (activator of cellulases 2), were given (Rauscher et al. 2006; Stricker et al. 2007b).

Interplay of Xyr1 with specific transcriptional regulators Ace1 and Ace2

In both xylanase promoters, the Xyr1-binding elements closely resemble the consensus sequences of the *H. jecorina* transcriptional regulator Ace1 (Saloheimo et al. 2000). In contrast to the first report on this transcription factor, Aro et al. (2003) demonstrated that a Δ*ace1* strain forms elevated levels of *xyn1* transcript on cellulose-based media. Distinct binding of Ace1 to an oligonucleotide corresponding to the part of the *xyn1* promoter, which contains the inverted repeat of two Xyr1-binding elements, was observed, and furthermore, electrophoretic mobility shift assays carried out with cell-free extracts, prepared from *H. jecorina* QM9414 mycelia grown on D-glucose, resulted in a complex of lower mobility compared to the cell-free extracts of *H. jecorina* QM9414 Δ*ace1* (Rauscher et al. 2006). As a summary, it was recently a competition between the Ace1 repressor and the Xyr1 activator for the Xyr1-binding elements (Rauscher et al. 2006) deduced. A similar mechanism has been shown for the regulation of the *alcA* gene of *A. niger*, where the transcriptional activator AlcR competes with CreA for the corresponding, partially overlapping binding sites (e.g. Marmorstein et al. 1992; Marmorstein and Harrison 1994; Mathieu and Felenbok 1994; Narendja et al. 1999).

As mentioned above, the transcriptional regulation of *xyn1* and *xyn2* strongly differs according to their respective inducer molecules; we currently follow the working hypothesis that in addition to a general inducer (Xyr1) specific repressors provoke these regulatory differences. Whereas a specific *xyn2*-repressing element (AGAA-box) has recently been identified (Würleitner et al. 2003), Ace1 acts as an antagonist of the Xyr1-driven *xyn1* gene transcription. This assumption is supported by the facts that vice versa neither a significant influence of Ace1 on *xyn2* expression (Aro et al. 2003) nor the occurrence of a functional AGAA-like element in the *xyn1* promoter can be observed (Rauscher et al. 2006). The detailed mode of interactions of Ace1 and Xyr1 with each other and with their corporate binding element (right motif of the Xyr1 element) in the *xyn1* promoter remains to be investigated.

Using deletion analysis, it was reported that a 55-bp fragment of the *xyn2* promoter (Zeilinger et al. 1996) contains all information necessary for regulating the *xyn2* gene expression. Further studies (Würleitner et al. 2003) using in vitro as well as in vivo strategies, identified nucleotide sequences within 55 bp being essential for the binding of proteins and being responsible for the *xyn2* regulation. It could be demonstrated that a protein–DNA complex related to both, basal transcription as well as induction of the *xyn2* expression, consists of at least the Hap2/3/5 complex (Zeilinger et al. 2001) and of Ace2 (Aro et al. 2001), contacting an undecameric motif 5′-GGGTAAATTGG-3′ (XAE; xylanase-activating element). The Hap2/3/5 complex contacts the CCAAT box within this motif. The CCAAT box, which is present in many fungal promoters (Brakhage et al. 1999), was shown to be involved in the maintenance of an active chromatin structure (Narendja et al. 1999). On the other hand, CCAAT boxes in filamentous fungi showing repressing functions (e.g. the *lysF* promoter [*A. nidulans*] and the *xyn2* promoter [*H. jecorina*]) have also been observed (Weidner et al. 2001; Würleitner et al. 2003). The induction-specific complex is counteracted by an uncharacterised DNA-binding protein (complex) binding to a 5′-AGAA-3′ box immediately upstream of the XAE (Stricker et al., manuscript in preparation). Whereas in a previous study, it was

shown that binding domains of Ace2 but not of the *A. niger* XlnR (van Peij et al. 1998b) contact this motif (Würleitner et al. 2003), recent investigations revealed binding of Xyr1 to the *H. jecorina* XAE (Stricker et al. 2007b). A probable explanation for this apparent contradiction could be a double amino acid exchange within the Zn-finger domain, comparing Xyr1 and XlnR proteins (Rauscher et al. 2006). Furthermore, it is noteworthy that only the Xyr1-binding elements in the *xyn1* promoter exactly match the XlnR otif (and are in vitro bound by Xyr1 as well as XlnR). This observation is in strict accordance with the fact that Xyr1 binds to all corresponding motives in both xylanase promoters, in contrast to Ace1, only contacting one part of the element in the *xyn1* but not in the *xyn2* promoter and Ace2 doing vice versa. Finally, it should be noted that no orthologue of Ace2 is present in the respective *Aspergillus* genomes, again strongly emphasising the significant differences in the transcriptional regulation of hydrolytic enzyme-encoding genes compared to *H. jecorina*.

In a research on the regulation of the production of hemicellulolytic enzymes in fungi, *H. jecorina* has—together with *Aspergillus*—the leading role. Significant progress has been made during the last few years in the identification and characterisation of in cis-acting elements and in trans-acting factors regulating xylanase expression. In *H. jecorina*, the general regulator of hydrolase formation (Xyr1) seems to be directly modulated in its mode of action by additional narrow domain transcription factors, such as Ace1 and Ace2. Although the binding elements are highly similar, DNA contact of these two factors is dedicated only to one of the promoters of the two major xylanolytic enzyme-encoding genes (i.e. Ace1 with p*xyn1* and Ace2 with p*xyn2*). First investigations revealed a spectrum of different mechanisms modifying the Xyr1-dependent transcriptosomes of the *xyn1* and *xyn2* genes, including competitions, homo- and heterodimerisations and the recruiting of additional regulatory proteins (for respective models, compare: Rauscher et al. 2006; Stricker et al. 2006; Stricker et al. 2007b; Würleitner et al. 2003).

In the case of the Ace1/Xyr1-driven *xyn1* expression, it is currently believed that Ace1 is a competitor of Xyr1 for only one of the two binding elements. A functional Ace1-repressor complex recruits an additional protein and also includes a single Xyr1. A dimer of Xyr1 is the basis for the induction-specific complex (Fig. 4; Rauscher et al. 2006).

Recent investigations on the role of Ace2 in the transcriptional regulation of the *xyn2* gene expression prompted to the working hypothesis that an intense interplay between Ace2 and Xyr1, including several steps of phosphorylation and probably heterodimerisation as well as the recruiting of additional proteins, is here also crucial for the formation of an active xylobiose-dependent *xyn2* transcriptosome.

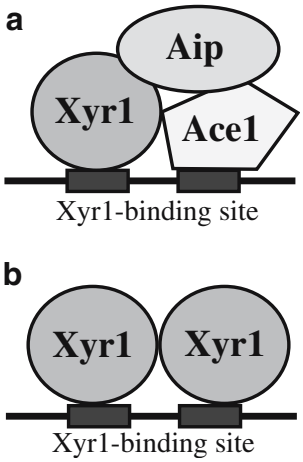

Fig. 4 A schematic presentation of the model of the involvement of transcriptional regulatory proteins, namely Ace1 (activator of cellulases 1) and Aip (Ace1-interacting protein, so far not further identified; Rauscher et al. 2006) in complex formation with the general activator Xyr1. The complexes are contacting the Xyr1-binding site (GGCTAA) arranged as an inverted repeat within the *xyn1* promoter of *H. jecorina* under repressing (glucose; **a**) and inducing (xylan; **b**) conditions

Concluding remarks

Amongst the numerous microorganisms exhibiting the capability of biomass degradation, the filamentous fungi *A. niger* and *H. jecorina* are impressive producers of hydrolytic enzymes, already applied in a series of industrial processes, e.g. food and feed industry (e.g. Galante et al. 1993; Grassin and Fauquembergue 1996; Poutanen 1997; Walsh et al. 1993), pulp and paper industry (e.g. (Buchert et al. 1998; Noé et al. 1986; Viikari et al. 1994) and textile industry (e.g. Koo et al. 1994; Pedersen et al. 1992).

Therefore, both fungi reached an extensive scientific attendance, not only according to their respective hydrolytic enzyme systems but recently also according to the regulation of expression of the corresponding encoding genes. Summing up, recent findings revealed major similarities but also striking differences in the transcriptional regulation of expression of these enzyme systems. The most obvious commonality is a general transcription factor (XlnR/Xyr1) signing responsible for transcriptional activation of the respective systems under all inducing conditions tested. Although such a uniformity of the main regulators points to highly similar induction mechanisms in both fungi, the opposite is the case. Whereas in *A. niger* the expression of all major cellulases and hemicellulases is co-regulated by the same inducer molecule (i.e. D-xylose), the induction mechanisms in *H. jecorina* are more diverse. At least four different inductor molecules (i.e. D-xylose, xylobiose, sophorose and lactose) have been described, but none of them has the potential to trigger the expression of all main cellulases and hemicellulases. In addition, some fine-tuning transcription factors (e.g. Ace1 and Ace2) were recently described to significantly interfere with the expression of individual hydrolases in *H. jecorina*. It is noteworthy that an orthologue of *ace2* could not be identified in the *A. niger* genome.

Evidence for another difference in the mode of action of the two main regulators, XlnR and Xyr1, is their interaction with

the DNA. Whereas in *A. niger* single in cis-acting elements were found in the 5′ upstream regulatory regions of the genes subject to regulation by XlnR, in *H. jecorina*, only double sites of the respective binding motif proved to be functional up to now. When using heterologously expressed DNA-binding domains, slight differences in the corresponding binding motives could be identified, probably because of a double-amino acid exchange within the zinc cluster region.

Nonetheless, comparing both proteins, high similarities were found, especially reflected in almost a 100% identity of various domains of the respective proteins. Recent analysis of some of these conserved regions in *A. niger* revealed their potential functionality.

Such investigations are indispensable prerequisites for a distinct understanding of the mode of action of those regulators and will in the future allow their genetic engineering, leading to strains with modified expression patterns of cellulases and/or hemicellulases. A potential strategy could follow either a constitutive or induced expression of modified versions of the regulatory proteins. For example, may the design of constitutively activated transcription factors lead to strains allowing inducer substance-independent enzyme productions.

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