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# Current Progress in the Analysis of Transcriptional Regulation in the Industrially Valuable Microorganism *Aspergillus oryzae*

Keiichi Nakajima, Motoaki Sano, and Masayuki Machida*

Molecular Biology Department, National Institute of Bioscience and Human-Technology, Higashi 1-1, Tsukuba, Ibaraki 305-8566, Japan

**Abstract** *Aspergillus oryzae* is considered to be an attractive host for heterologous protein production because of its safety and ability to secrete large amounts of proteins. In order to obtain high productivity, thus far promoters of amylases have been most widely used in *A. oryzae*. Recent progress in cloning and expression analysis, including EST sequencing, revealed that glycolytic genes represent some of those most strongly expressed in *A. oryzae*. Therefore, promoters of glycolytic genes could be important alternatives to promoters of amylases because lower amounts of proteases are produced in the presence of glucose. Several *A. oryzae* transcription factors responsible for the induction and/or maximum expression of many industrially important genes encoding amylases and proteases have been cloned and characterized. In addition to the transcriptional regulatory factors, the gene encoding the largest subunit of RNA polymerase II, constituting the basic transcription machinery, has also been cloned from *A. oryzae*. This recently acquired understanding of the details of transcriptional regulatory mechanisms and factors will facilitate engineering flexible controls for the expression of proteins important for the fermentation industries.

*Keywords*: *Aspergillus oryzae*, transcriptional regulation, transcriptional regulatory factor, promoter

## INTRODUCTION

A filamentous fungus, *Aspergillus oryzae*, has played an important role in Japan for many years in the fermentation industries producing soy sauce and miso (soybean paste), as well as sake (alcoholic beverage). In addition to the traditional uses, *A. oryzae* is considered an attractive host for heterologous protein production for the following reasons: First, *A. oryzae* is considered to be safe. Because it has been used in food industries for a long time, this organism is listed GRAS (generally recognized as safe) by the Food and Drug Administration (FDA) of the USA. Second, *A. oryzae* has the ability to secrete large amounts of enzymes such as amylases, proteases and nucleases. The development of molecular genetic techniques including DNA transformation systems has made it possible to overproduce many extracellular proteins [1] including aspartic protease (*Rhizomucor miehei*) [2], lipases (*R. miehei*) [3], thaumatin (*Thaumatococcus daniellii*) [4], lactoferrin (human) [5], chymosin (calf) [6] and lysozyme (human) [7] by this organism.

In order to increase protein productivity, the use of a promoter with strong transcriptional activity is generally most promising and effective. Therefore, strong promoters from the genes encoding industrially important enzymes with high levels of expression have been extensively analyzed to date. Alternatively, the improvement of the expression level of the target gene may be achieved by increasing the number of expression cassettes, each of which is composed of a promoter and the gene to be expressed. Productivity from the transformant having multiple copies of the cassette rises depending on the increasing number of cassettes in the genome. Increasing the number of regulatory elements (*cis*-elements) in the promoter instead of increasing the number of cassettes may also be effective provided that detailed knowledge of these regulatory elements is available [8]. However, in either case, production levels from strains possessing excess copies of the transcriptional regulatory element (on average, more than 5-6 copies) did not increase further in response to increasing the number of cassettes or regulatory elements [1,8]. Furthermore, the expression of other genes (perhaps regulated in a similar way by the promoter used for the over-expression), may be significantly reduced, probably because of exhaustion of the regulatory factors within the cell.

Many species of *Aspergillus* produce extracellular proteases, which are utilized for protein degradation in the fermentation industries. Strains with high protease production have been sought for many years to enhance productivity in soy source manufacture. On the other hand, protease degrades the protein to be produced

*Corresponding author
Tel: +81-298-61-6214 Fax: +81-298-61-6240
e-mail: machida@nibh.go.jp

from the engineered strain. To date, therefore, many attempts have been made to reduce protease activities by classical mutagenesis. The disruption of transcription factors necessary for the expression of protease is efficient in this respect, as well as disruption of the protease structural gene itself. Therefore, understanding the transcriptional regulatory mechanisms in detail, including the factors regulating transcription, will facilitate the engineering of flexible control of the expression of enzymes important in the fermentation industries.

In this paper, we focus on the transcriptional regulation of *A. oryzae* from the viewpoint of the fermentation industries and biotechnological applications. In the first part of the manuscript, the promoters and highly expressed genes, the promoters of which are thought to have strong promoter activity, are discussed. While amylase promoters from *amyB*, *glaA* and *agdA* have been almost exclusively used for gene expression in *A. oryzae*, the promoters of the glycolytic genes (*gpdA* and *pki*) have been widely used in *Aspergillus nidulans* and *Aspergillus niger* [9,10]. Thus, the promoters of glycolytic genes recently cloned from *A. oryzae* could be another choice for the strong expression of foreign genes. In the second part of the manuscript, transcription factors important for the expression of the strong promoters and of industrially important genes are discussed.

## STRONG AND INDUCIBLE PROMOTERS FROM AMYLASE GENES

The amylase promoters are among the strongest and best characterized promoters from *A. oryzae*. These promoters are known to be inducible by oligosaccharides such as maltose, but are repressed by glucose in the medium [11]. In order to overproduce proteins, the target gene is introduced downstream of the promoter which has desirable expression characteristics. Inducible promoters such as the amylase promoters are advantageous for the production of proteins that are either toxic or instable. In *A. oryzae*, promoters from amylases (*amyB, glaA*) have been well-studied and are most widely used for strong and inducible expression (19-fold and 15-fold induced in the presence of maltose, respectively) [11].

A comparison of the promoter sequences of the *A. oryzae* amylase genes, *amyB*, *glaA* and *agdA*, revealed four highly conserved sequences in each promoter, which were designated regions I, II, IIIa, and IIIb [12]. Deletion analysis using *Escherichia coli uidA* as a reporter showed that region IIIa was involved in both high expression and maltose induction and that region I and region IIIb were involved in enhancing expression in conjunction with region IIIa [12-14].

Introduction of 12 tandem repeats of region III into the *agdA* promoter enhanced the promoter activities 5- and 18-fold as compared to the intrinsic promoter in the presence of maltose and glucose as a carbon source, respectively [8]. In practice, α-glucosidase activity from the transformant carrying a single copy of *agdA* under the control of an improved promoter showed more than 70-fold the activity of the recipient strain in the presence of glucose, and the transformant possessing mutiple copies of the cassette showed a 140-fold increase. Interestingly, Taka-amylase and glucoamylase were strongly repressed in the α-glucosidase-overproducing transformant, which harbored multiple copies of the region III regulatory element [8]. The promoters of *amyB* and *glaA*, which encode Taka-amylase and glucoamylase, respectively, also contained an element homologous to region III. Recently, reduction of productivity of the amylases was shown to be due to exhaustion of the common *trans*-acting factor, amyR, which specifically binds to the consensus sequence found in the region III [15]. The binding sequence of CreA, which mediates catabolite repression in *Aspergillus*, is also present in the *amyB* promoter [16]. A CCAAT sequence found in region IIIb may also be a *cis*-acting element, to which an *A. oryzae* AnCP-like protein binds [17,18].

### Production of Industrially Important Enzymes by using Amylase Gene Promoters of *A. oryzae*

Several attempts to overexpress valuable proteins to nearly industrial level have been reported using amylase promoters. *R. miehei*, *Humicola lanuginosa* and *Candida arctica* lipases were overproduced using *A. oryzae* as a heterologous host. The main use of industrial fungal lipases is as additives in washing detergents and in the food industries. Lipases for washing powder are partly produced by recombinant strain of *A. oryzae* [19], in which *R. miehei* lipase cDNA was inserted between the *A. oryzae* α-amylase gene promoter and the *A. niger* glucoamylase gene terminator [3]. *R. miehei* aspartic protease was also successfully produced by using the same expression system, yielding greater than 3 g/L of the protease secreted in culture medium [2].

Other attempts to overexpress valuable proteins to nearly industrial level have been made using the glucoamylase promoters. *A. oryzae* nuclease S1, which has been widely used for removal of single-stranded DNA in genetic manipulation, was overexpressed by using the *glaA* promoter. The transformant secreted approximately 100-fold nuclease S1 originally produced by the recipient strain without the expression cassette of the enzyme [20]. Calf chymosin was successfully expressed from its cDNA and secreted into culture medium under control of the *glaA* promoter [6]. Higher level secretion of calf chymosin (150 mg/kg wheat bran) was achieved by the fusion of the chymosin cDNA to the *A. oryzae* glucoamylase gene and with the wheat bran substrate culture [21].

## GLYCOLYTIC GENES AS DONORS OF STRONG PROMOTERS

### Expression of Glycolytic Genes

Some of the glycolytic genes such as phosphoglycer-

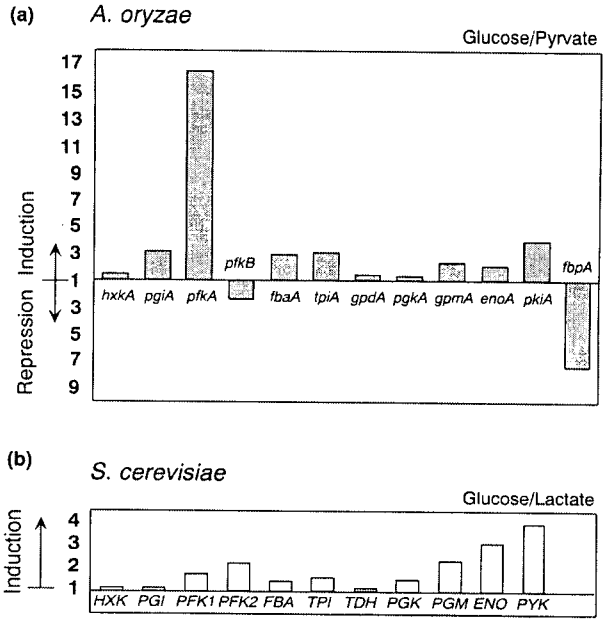


Fig. 1. Regulation of *A. oryzae* and *S. cerevisiae* glycolytic genes by glucose. (a) The mRNA levels from mycelia grown in medium containing glucose or pyruvate are compared. The values greater than 1 on the vertical axis indicate induction rates by glucose while the values smaller than −1 indicate repression rates [24]. (b) The mRNA levels from *S. cerevisiae* cells grown in medium containing glucose or lactate are compared, quoted from Moore *et al.* [95].

ate kinase and enolase genes from yeast and filamentous fungi are known to have promoters with strong expression. Generally, the glycolytic enzymes constitute 30-60% of cellular protein. *A. oryzae* glycolytic genes are also highly expressed in the presence of glucose, as has also been found in yeast. The level of expression of *A. oryzae enoA* encoding one of the glycolytic enzymes, enolase [22], reached approximately 3% of total mRNA when induced by glucose. This level is comparable to that of Taka-amylase, which is known to be one of the most highly expressed inducible genes [23]. As expected, glycolytic genes were found frequently in the *A. oryzae* ESTs (expressed sequence tags) (Hagiwara *et al.* unpublished data, URL http://www.aist.go.jp/RIODB/ffdb/index.html). These results suggest that the promoters of *A. oryzae* glycolytic genes are important for industrial applications.

Recently, all the glycolytic genes were cloned and sequenced. With the exception of *pfkB* and *fbpA*, most of the *A. oryzae* glycolytic genes were induced to different extents by glucose [24](Sano *et al.* in press). The genes most highly induced by glucose were phosphofructokinase (*pfkA*) and pyruvate kinase (*pkiA*), which were induced 16- and 4-fold, respectively. Interestingly, the expression profile of *A. oryzae* glycolytic genes was very similar to that of *Saccharomyces cerevisiae* (Fig. 1). A pyruvate decarboxylase gene, *pdcA*, which is probably one of the most highly expressed genes in *A. oryzae*, was recently cloned and sequenced [25]. The expression of *pdcA* was also strongly induced in the presence of glucose (unpublished data).

## Promoters of Glycolytic Genes

Promoters repressed by glucose, such as the amylase promoters, may sometimes be unsuitable for protein production in glucose-containing media. However, expression in glucose would be preferable for industrial applications because lower amounts of proteases are produced by fungi in the presence of glucose compared to media containing complex plant material [26]. Thus, the promoters of glycolytic genes with strong and glucose-inducible expression could be an important avenue to pursue.

Most of the yeast glycolytic genes are co-regulated by the common transcriptional regulatory genes, *gcr1* and *gcr2* [27-31]. Further, many house-keeping genes includeing glycolytic genes are regulated by the general transcriptional regulatory factor, Rap1p [32-34]. Gcr1p and Rap1p directly bind to their recognition sequences, CT-blocks and RPG-boxes, respectively, which exist in the 5′-flanking region of most of the glycolytic genes, resulting in glucose-dependent expression [35-42]. However, an RPG-box was not found in the 5′-flanking region of the *A. oryzae enoA* [43] and *pgkA* (Ogawa *et al.*, GenBank D28484) genes. Further, the sequence-specific DNA-binding activity that recognized the yeast RPG-box (Rap1p-binding consensus) could not be detected in *A. oryzae* whole cell extracts; instead, a factor that specifically bound to a short element free of Rap1p- binding consensus in the *enoA* upstream region was detected in the same extract (unpublished data). These results suggest that the mechanism of transcriptional regulation in *A. oryzae* glycolytic genes is different from that in *S. cerevisiae*, although the expression profiles of the glycolytic genes resemble one another.

The 5′-flanking region of *A. oryzae enoA* and *pgkA* contained a CT-rich region, which is also found in the promoter of various highly expressed genes of filamentous fungi [44]. There are two highly conserved elements designated *pgk* and *gpd* boxes, which were identified by sequence comparison among *pgkA* and *gpdA* promoters from *A. nidulans* and *A. niger*. The *A. oryzae pgkA* promoter has a sequence homologous to *pgk* box, which is involved in carbon source regulation [45]. Both *pgk* and *gpd* boxes were found in the 5′-flanking region of *A. oryzae gpdA* (unpublished data) as in the *A. nidulans gpdA* promoter [46,47]. Deletion of *gpd* box over an approximately 50 bp length resulted in a two- to threefold reduction in expression as assessed using promoter-reporter gene fusion in *A. nidulans* [46,47]. Detailed analysis revealed that 18 bp at the 3′ end of the *gpd* box was essential for the transcriptional enhancement activity in the box [48]. The *A. nidulans gpdA* promoter has been widely used in vector constructions for heterologous gene expression in fungi. The intracellular heterologous protein expressed by this promoter reached 10-25% of total soluble proteins [10]. Despite

Table 1. Experimentally characterized transcription regulatory factors from *A. oryzae*

| Transcription regulatory factors | Property | Genes under control[a] | Amino acid identity to the *A. nidulans* homologue | Homologous genes[b] | DNA-Binding domain | References |
|---|---|---|---|---|---|---|
| AmyR | Positive regulator of amylase genes | *agdA, amyB, glaA* | 62.3% | *MALR* | $C_6$ type zinc finger | [15,49] |
| Area | Positive regulator of nitrogen metabolism genes | Nitrogen metabolism genes | 70.0% | *GAT1* | $C_4$ type zinc finger | [52] |
| FacB | Positive regulator of acetate utilization genes | (*acuD*), (*acuE*), (*amdS*), (*facA*) | 68.9% | *CAT8* | $C_6$ type zinc finger | [54] |
| AmdR | Positive regulator of omega amino acid and lactam utilization genes | (*amdS*), (*gabA*), (*gatA*), (*lamA/B*) | 70.9% | - | $C_6$ type zinc Finger | [58] |
| PacC | Regulator of pH inducible Genes | (*gabA*), (*ipnA*), (*pacA*), (*palD*), (*prtA*) | 72.3% | *RIM1* | $C_2H_2$ type zinc finger | unpublished data |

[a] The genes in parentheses are deduced from the results by *A. nidulans*.
[b] Homologus genes from *S. cerevisiae* having an E value smaller than $10^{-20}$ in the BLASTX search.

wide use for heterologous gene expression and extensive analyses, no data on transcriptional regulatory factors have been reported for the *A. nidulans gpdA* promoter. Because *A. oryzae pgkA* and *gpdA* were frequently observed in ESTs, promoters from those genes are expected to be very strong and useful for heterologous gene expression.

## TRANSCRIPTIONAL REGULATORY FACTORS FROM *A. ORYZAE*

Whereas there has been relatively little work on the general transcriptional machinery in *Aspergillus* in comparison with yeast, increasing numbers of transcriptional regulatory proteins have been identified in *A. oryzae*. Most *A. oryzae* genes encoding transcription factors except *amyR* [49] were cloned by hybridization using the corresponding *A. nidulans* gene as a probe. Because the *A. nidulans* genes were initially isolated by complementation using mutants, much genetic data and information on the phenotypes of the mutants has been accumulated in this species. Transcriptional regulatory factors from *A. oryzae*, which have been experimentally examined to date are summarized in Table 1.

### Transcriptional Regulatory Factors from *A. oryzae*

1) AmyR [15,49]

Recently, the transcriptional activation factor responsible for maltose induction of the amylase genes was isolated from *A. oryzae* by the complementation of a regulatory mutant of amylases [49] and by the reversion of the reduced expression of *amyB* in the transformant having mutiple copies of region III [15]. The isolated gene was designated AmyR. The *amyR* gene encodes a 604-amino acid protein containing a GAL4-type zinc finger motif at the N-terminus.

Searches in the databases showed that the central part of AmyR (residues 242-492) can be aligned with regulatory proteins involved in maltose utilization including MALR [50] from *S. cerevisiae* and SUC1 [51] from *Candida albicans*, which are involved in both maltose and sucrose utilization. These two proteins also contain a GAL4-type zinc finger cluster highly homologous to the one found in AmyR. The DNA-binding domain of AmyR binds to two types of sequences found in a number of promoters. One type of binding site is characterized by two CGG triplets separated by eight nucleotides, and the other type has only one CGG triplet which is followed by the sequence AAATTTAA.

2) AreA [52,53]

The *areA* gene encodes the major regulatory protein which activates transcription of many structural genes encoding enzymes for nitrogen source catabolism under conditions of limited nitrogen availability. The *areA* gene of *A. oryzae* was cloned by cross-hybridization with the *A. nidulans areA* gene and was found to encode an 866-amino-acid protein that is very similar to other fungal nitrogen regulatory proteins. The *areA* protein contains a single $CX_2CX_{17}CX_2C$ DNA binding motif which recognizes and binds to DNA sequences containing a core 5'-GATA-3' sequence. Functional analyses indicated that the N-terminal region of the *A. oryzae* AreA protein was dispensable for function and revealed a probable acidic activation domain in the protein. C-terminal truncation of the protein resulted in derepression of several nitrogen-controlled activities in *A. nidulans*, while deletions extending into the con-

served GATA-type zinc finger region abolished the activator function. The DNA binding domain and extreme C-terminal residues of these proteins are highly conserved.

In *A. oryzae areA* mutants, the NADP-glutamate dehydrogenase levels were reduced, whereas the glutamine synthetase levels were not affected. The AreA protein may play an important role in the regulation of nitrogen assimilation in addition to its previously established regulatory role in nitrogen catabolism.

3) FacB [53,54]

The *facB* gene of *A. oryzae* encodes the major regulator of genes involved in acetate utilization. Sequencing of the *facB* gene revealed that it encodes a protein that contains an N-terminal GAL4-like Zn(II)2Cys6 binuclear cluster for DNA binding, leucine zipper-like heptad repeat motifs and central and C-terminal acidic α-helical regions, consistent with a function as a DNA-binding transcriptional activator. The Zn(II)2Cys6 cluster shows strong similarity with those of the *S. cerevisiae* carbon metabolism regulatory proteins CAT8 [55] and SIP4 [56]. A significant level of similarity with CAT8 is found throughout the length of the protein, suggesting at least partial functional homology. The FacB-binding sequences derived from the footprints are TCC/$GN_{8\text{-}10}$C/GGA and GCA/C $N_{8\text{-}10}$T/GGC. Both binding sites show imperfect rotational symmetry. In *A. nidulans, facB* mutants isolated by resistance to fluoroacetate, grew poorly on acetate as a sole carbon source and showed decreased levels of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase (FBP) [57].

4) AmdR [58]

The *amdR* gene encodes a positively acting regulatory protein which mediates omega amino acid induction of the *amdS, gatA* (GABA transaminase), and *gabA* (GABA permease) genes. The *amdR* gene contains a $Zn(II)_2Cys_6$ DNA-binding domain at the N-terminus, and four activation domains in the middle and at the C-terminus which are required for full activation. The *amdR* inactivated mutant resulted in inability to grow on GABA as a carbon and/or nitrogen source(s) indicating that GABA utilization is *amdR*-dependent in *A. oryzae* as it is in *A. nidulans.*

5) CreA [59]

The *creA* gene encodes a wide-domain regulatory protein mediating carbon catabolite repression of many genes such as polysaccharide hydrolysis, ethanol metabolism and proline utilization [60-62]. CreA has two zinc-finger regions of the Cys2His2 class, which bind to a 5′-SYGGRG-3′ target sequence, and bear strong similarity to the *S. cerevisiae* Mig1p [63] glucose repressor protein. CreA inhibits transcription of many target genes by binding to the specific sequences in the promoter. The *creA* gene has not been cloned from *A. oryzae* to date. The DNA-binding domain of *creA* expressed as a fusion protein with maltose-binding protein could bind to the *A. oryzae* amylase promoter[16].

Consensus sequences for the binding of *A. nidulans* CreA (5′-SYGGRG-3′) were found in the 5′-flanking region of the *A. oryzae amyB* and *fbpA* genes, expression of which was repressed in the presence of glucose.

6) PacC

PacC is a wide-domain regulatory factor implicated in modulating gene expression in response to ambient pH [64-66]. *pacC* itself is an alkaline-expressed gene, subjected to autogenous transcriptional activation, amplifying the alkaline ambient pH signal. PacC protein possesses three elements which resemble zinc fingers of the $C_2H_2$ class and bind to GCCARG promoter sites. Recently, the *pacC* gene was cloned from *A. oryzae* (Sano *et al.*, unpublished data).

## CCAAT-binding Protein

The CCAAT motif, which is found in many fungal promoters as well as in higher eukaryotes, is also present in many *A. oryzae* promoters including the Taka-amylase promoter. A complex designated HAP was first shown to bind to the CCAAT sequence in *S. cerevisiae* [67]. In recent years, HAP-like complexes have been identified in filamentous fungi. CCAAT-binding factors of *A. nidulans* have been independently found to bind to the acetamidase (*amdS*), Taka-amylase (*amyB*) and isopenicilline N-acyltransferase (*aat*) genes, and were designated *A. nidulans* CCAAT factor (AnCF) [68], *A. nidulans* CCAAT-binding protein (AnCP) [17] and penicillin-regulatory protein (PENR1) [69], respectively. Three CCAAT-binding complexes appeared to be the same, and AnCF/AnCP were counterparts of the *S. cerevisiae* HAP complexes [70]. Recently, HAP-like complexes were also found in *A. oryzae* [71]. *In vivo* determination of the *A. oryzae* α-amylase activity in *A. nidulans* was used as a reporter system to measure the effects of directed mutagenesis of the CCAAT box. When the CCAAT sequence was mutated to CGTAA, *amyB* expression was reduced to 30% of that observed with the wild-type construct. Further mutagenesis of this motif made binding of AnCP impossible and abolished its expression when using *A. nidulans* as an intermediate host [18]. These results illustrate the positive regulatory function of the CCAAT motif confers on *amyB* expression [18]. Region IIIb, com-prising a CCAAT sequence, is also likely to be a general *cis*-acting element.

## Basal Apparatus for Transcription from *Aspergillus*

Studying the elements and factors important for transcriptional regulation of each gene provides useful information to improve promoter activity and characterize the organism. However, understanding the basic apparatus for transcription as a common regulatory machinery affected by all the transcription factors is also important. While transcriptional regulatory factors have been studied regarding expression, little is known

of the basal transcriptional apparatus, RNA polymerase II (polII) and general transcription factors (GTFs) in any *Aspergilli*. There are reports only on RNA polymerase II (polII) and TATA-box binding protein (TBP) [72,73]. TBP isolated from *A. nidulans* was functional in *S. cerevisiae*. The polypeptide components of polII and motifs of GTFs are well-conserved among eukaryotes from yeast to human. Therefore, the other components of *Aspergillus* basal transcriptional apparatus are thought to be functionally compatible with yeast.

Recently, the largest subunit of RNA polymerase II (polIII) was cloned from *A. oryzae*, which was the first example of polIII cloned from a filamentous fungus. As a distinguishing feature, polIII possesses a carboxyl-terminal domain (CTD) composed of heptapeptide repeats (YSPTSPS). Deletion studies demonstrate that CTD is essential for cell growth in mouse and *S. cerevisiae* [74-76]. CTD is known to interact directly with the basal transcription factors, TATA-box binding protein (TBP) [77] and with mRNA processing factors [78]. Although the numbers of repeats in CTDs from yeast and *A. oryzae* polIIILs were similar (25-29 repeats) (Fig. 2) [79,80], the amino acid sequence of the repeats in *A. oryzae* polIIIL was much less conserved than in yeast polIIIL.

The compatibility of polIIILs and CTDs from several different organisms has been examined using yeast mutants lacking polIIIL function. Mouse polIIIL [81] and yeast polIIIL having hamster CTD instead of their original CTD [76] successfully complemented the *rpb1* phenotype, but yeast polIIIL, the CTD of which was replaced by *Drosophila* polIIIL CTD [76], and *A. oryzae rpbA* (unpublished data) failed to do so. The low level of conservation of heptapeptide repeats in *A. oryzae* polIIIL as in *Drosophila* polIIIL, especially at positions 1, 2 and 5 which were important for the viability of yeast cells [82,83], may be functionally distinguished from polIIILs from other species that contain highly conserved heptapeptide repeats.

In recent years, novel glycosylated proteins modified by *N*-acetylglucoxamine nomosaccharide through *O*-glycosidic linkage (*O*-GlcNAc) have been demonstrated in various eukaryotic organisms [84] although most of the *O*-GlcNAc-modified protein was found in higher eukaryotes. Unlike other glycoproteins, the *O*-GlcNAc-bearing proteins have been found to be localized in the nuclear and cytoplasmic compartments of the cell [85,86]. Many transcription factors for genes transcribed by RNA polymerase II, including CTD of mammalian polIII, were found to be involved in this class of modification [87-90]. It has been shown that *O*-GlcNAc turns over more rapidly than the protein itself [91,92]. It was therefore postulated that *O*-GlcNAc is a regulatory modification analogous to phosphorylation. The fact that the human transcription factor, SP1, requires *O*-GlcNAc modification for the transcription enhancement activity *in vitro* strongly suggests that *O*-GlcNAc is one of the key regulatory modifications for transcription by RNA polymerase II [93]. Interestingly, several DNA-binding proteins that are modified by *O*-GlcNAc have been detected in lower eukaryotes, such as *A. oryzae* [94].

| Mouse | *Drosophila* | *S. pombe* | *S. cerevisiae* | *A. oryzae* |
|---|---|---|---|---|
| YSPTSPA | YSPTSPN | YGLTSPS | FGVSSPG | YSPSSPFS |
| YEPRSPGG | YTASSPGG | YSPSSPG | FSPTSPT | ANPTSPG |
| YTPQSPS | ASP---N | YSTSP-A | YSPTSPA | YSP-SSS |
| YSPTSPS | YSPSSPN | YMPSSPS | YSPTSPS | YSPTSPG |
| YSPTSPS | YSPTSPL | YSPTSPS | YSPTSPS | MAMTSPR |
| YSPTSPN | Y---ASPR | YSPTSPS | YSPTSPS | FSMTSPG |
| YSPTSPS | YASTTPN | YSPTSPS | YSPTSPS | FSPASPS |
| YSPTSPS | FNPQSTG | YSPTSPS | YSPTSPS | FAPTSPA |
| YSPTSPS | YSPSSSG | YSATSPS | YSPTSPS | YSPTSPAYGQ |
| YSPTSPS | YSPTSPV | YSPTSPS | YSPMSPS | ASPTSPS |
| YSPTSPS | YSPTVQ- | YSPTSPS | YSPTSPS | YSPTSPG |
| YSPTSPS | FQSSPS- | YSPTSPS | YSPTSPS | FSPTSPN |
| YSPTSPS | FAGSGSNI | YSPTSPS | YSPTSPS | YSPTSPS |
| YSPTSPS | YSPGN-A | YSPTSPS | YSPTSPS | FSPASPA |
| YSPTSPS | YSPSSSN | YSPTSPS | YSPTSPS | FSPTSPS |
| YSPTSPS | YSPNSPS | YSPTSPS | YSPTSPS | YSPTSPA |
| YSPTSPS | YSPTSPS | YSPTSPS | YSPTSPA | IGGAARH |
| YSPTSPS | YSPSSPS | YSPTSPS | YSPTSPS | LSPTSPTSPK |
| YSPTSPS | YSPTSPC | YSPTSPS | YSPTSPS | YTPTSPG |
| YSPTSPS | YSPTSPS | YSPTSPS | YSPTSPS | WSPTSPQT |
| YSPTSPS | YSPTSPN | YSPTSPS | YSPTSPN | YSPTSPN |
| YSPTSPN | YTPVTPS | YSPTSPS | YSPTSPN | FAGSPTSP |
| YSPTSPN | YSPTSPN | YSPTSPS | YSPTSPS | GGPTSPG |
| YTPTSPS | YS-ASPQ | YSPTSPS | YSPTSPG | YSPTSPA |
| YSPTSPS | YSPASPA | YSPTSPS | YSPGSPA | FSPSSPRQ |
| YSPTSPN | YSQTGVK | YSPTSPS | YSPKQDE | |
| YTPTSPN | YSPTSPT | YSPTSPS | QKHNENENSR | |
| YSPTSPS | YSPPSPS | YSPTSPS | | |
| YSPTSPS | YDGSPGSPQ | YSPTSPS | | |
| YSPTSPS | YTPGSPQ | | | |
| YSPSSPR | YSPASPK | | | |
| YTPQSPT | YSPTSPL | | | |
| YTPSSPS | YSPSSPQ | | | |
| YSPSSPS | HSP-SNQ | | | |
| YSPTSPK | YSPTGST | | | |
| YTPTSPS | YSATSPR | | | |
| YSPSSPE | YSPNMSI | | | |
| YTPASPK | YSPSSTK | | | |
| YSPTSPK | YSPTSPT | | | |
| YSPTSPK | YTPTARN | | | |
| YSPTSPT | YSPTSPM | | | |
| YSPTTPK | YSPTAPSH | | | |
| YSPTSPT | YSPTSPA | | | |
| YSPTSPV | YSPSSPT | | | |
| YTPTSPK | FEESEDVRKGGRG | | | |
| YSPTSPT | | | | |
| YSPTSPK | | | | |
| YSPTSPT | | | | |
| YSPTSPKGST | | | | |
| YSPTSPG | | | | |
| YSPTSPT | | | | |
| YSLTSPA | | | | |
| ISDEEN | | | | |

Fig. 2. Comparison of PolIIILs CTD. The numbers of the heptapeptide repeats in CTD (YSPTSPS) are 52, 45, 29, 26 (or 27) and 25 times in mouse, *Drosophila*, *Schizosaccharomyces pombe*, *S. cerevisiae* and *A. oryzae* polIIILs, respectively. The repeats perfectly matched to the consensus sequence are indicated by black boxes[96].

## Conclusion and Outlook

*A. oryzae* is a very important microorganism for the fermentation and biotechnology industries. Lack of basic knowledge and incompatibility in genetics have made the extensive use of this organism difficult. Recent development of technologies for genetic engineering and for genetic analysis are reducing this disadvantage and rendering this organism more attractive. Studying transcriptional regulation is one of the most important contributors to this situation. However, available information on transcriptional regulation, including *cis*-elements and *trans*-acting factors of *A. oryzae*, is still insufficient for the extensive use of this organism. Genomic research including large scale EST analysis will accelerate the accumulation of basic knowledge of this

organism. Most of the transcription factors found in *A. oryzae* to date show high homology in amino acid sequence to yeast factors with similar functions. However, the factor(s) or their recognition sequence(s) for the regulation of glycolytic genes appear different in the two organisms. EST and genome sequencing of *Aspergilli* will be of great importance not only for industrial applications but also for the elucidation of the differences in transcriptional regulatory mechanisms between filamentous fungi and yeast.

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