Paracoccidioides brasiliensis RNA biogenesis apparatus revealed by functional genome analysis

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ABSTRACT. The RNA biogenesis machinery of *Paracoccidioides
brasiliensis* was assessed by comparative analyses of PbAESTs (*P.
brasiliensis* assembled expressed sequence tags (ESTs)) with sequences
from *Saccharomyces cerevisiae* MIPS database. PbAESTs related to
almost all categories of *S. cerevisiae* RNA biogenesis were found. Two
of the 12 *S. cerevisiae* RNA Pol II core subunits, Rpb3 and Rpb7, were
found, probably reflecting the growth phase from which the cDNA li-
braries used in ESTs generation were constructed, as well as the low
abundance of some of these transcripts. We have also found orthologs
to TATA-box-binding protein (TBP), and at least one subunit of each
TBP-associated factors (TFII) in *P. brasiliensis* transcriptome, except
TFIIB. Genes associated to the chromatin remodeling complex, as well
as transcription factors probably involved in the control of genes associ-
ated to a sexual cycle and virulence, were also identified. With respect
to the pre-mRNA processing, 65 PbAEST orthologs to *S. cerevisiae*

basal splicing machinery and 21 orthologs of 5'- and 3'-end formation processes were found. Components involved in RNA interference were detected, suggesting that this gene expression regulation mechanism is probably used by *P. brasiliensis*. Twelve PbAESTs related to Pol I and Pol III machineries were assigned as *S. cerevisiae* orthologs. Finally, 25 and 10 PbAESTs associated to rRNA and tRNA processing, respectively, were detected. Taken together, our results enable us to depict, for the first time, a global view of transcription and RNA processing in *P. brasiliensis*.

Key words: *Paracoccidioides brasiliensis*, Transcriptome analysis, Expressed sequence tag, Transcription, RNA modifications, Splicing

INTRODUCTION

The human pathogenic fungus *Paracoccidioides brasiliensis* is the etiological agent of paracoccidioidomycosis, the most prevalent mycosis in Latin America (Franco, 1987; Restrepo et al., 2001). Besides the importance of this disease due to its high prevalence in endemic rural areas, where it has been estimated to affect around 10 million individuals, it also serves as a model of infection by other intracellular pathogens. Paracoccidioidomycosis is an interesting model for the understanding of host-pathogen interactions common to other chronic pulmonary disorders, such as tuberculosis, criptococcosis, histoplasmosis, and candidiasis, among others.

A remarkable feature common to several pathogenic fungi is their ability to differentiate from mycelium to yeast morphologies or vice-versa (Gow et al., 2002). *P. brasiliensis* is found as mycelia at 22-26°C and as yeasts at 37°C, or upon host tissue invasion.

In association with other laboratories from the central region of Brazil, we have carried out an expressed sequence tag (EST) genome project entitled "Functional and Differential Genome of the Fungus *Paracoccidioides brasiliensis*" (http://www.biomol.unb.br/Pb), as described by Felipe et al. (2003). Single-pass 5' sequencing from non-normalized cDNA libraries of mycelium and yeast cells generated a total of 19,718 high-quality ESTs. Upon CAP3 assembly, 2,655 contigs and 3,367 singlets, which constitute the so-called 6,022 PbAESTs (*P. brasiliensis* assembled ESTs) database was generated (Felipe et al., 2005). We have analyzed the RNA biogenesis of *P. brasiliensis*, comparing our transcriptome findings with those of *S. cerevisiae*, which is considered an important eukaryotic model to describe the transcription machinery. Since the *S. cerevisiae* genome has the most complete annotation available, we have used available *S. cerevisiae* MIPS databases to perform the *P. brasiliensis* transcriptome annotation of transcription-related compounds. We have found representatives of almost all categories of *S. cerevisiae* RNA synthesis and processing machineries, resulting in a clear overview of the genes that participate in *P. brasiliensis* gene expression. However, it is important to notice that probably many of the basal and/or regulated factors associated to these processes could be underscored, since some of them probably are *P. brasiliensis*-specific, as well as could be represented by transcripts of low copy number.

PARACOCCIDIOIDES BRASILIENSIS mRNA TRANSCRIPTION

Transcription of eukaryotic protein-encoding genes depends on the cooperative interaction of the RNA polymerase II (Pol II) core enzyme with various transcription factor complexes, including general transcription factors (GTFs), chromatin-modifying complexes and several other specific regulatory factors (Mitsuzawa and Ishihama, 2004).

Messenger RNA transcription in eukaryotic systems is a very complex process, which involves a great number of proteins working in a synchronized manner (reviewed by Howe, 2002). After chromatin remodeling and transcription pre-initiation complex (PIC) formation, the RNA Pol II core enzyme, associated to several GTFs, is recruited to the promoter, assembling a functional transcription initiation complex and starting the nascent pre-mRNA synthesis. Following promoter clearance, the elongation step, responsible for the bulk of transcription, is achieved. Differently from prokaryotic transcription termination, where precise terminating sites are defined, the eukaryotic transcription termination is tightly coupled to the 3'-end mRNA formation process (Proudfoot et al., 2002). Following termination, RNA Pol II, and the associated protein factors, are dissociated from the DNA template, releasing the nascent transcript and allowing the polymerase to bind to another promoter (Lee and Young, 2000). During and after transcription, nascent mRNA molecules are targets of a series of modifications known as RNA processing (Proudfoot et al., 2002). These modifications include addition of a 5' cap, removal of intron sequences by splicing and 3' polyadenylation. The mature mRNA is then ready to be transported to cytoplasm, where it can be translated into polypeptides.

RNA POLYMERASE II CORE

RNA Pol II, the enzyme responsible for the expression of all protein-coding genes and a few non-coding RNAs in eukaryotes, is highly conserved from yeasts to humans (Sakurai et al., 1999; Hahn, 2004). In *S. cerevisiae*, RNA Pol II consist of 12 subunits, named Rpb1 to Rpb12, whose genes are all required for normal cell growth (Lee and Young, 2000).

Rpb1, the largest subunit of RNA Pol II, contains a carboxy-terminal repeat domain (CTD), not shared by other RNA polymerases, that consists of tandem repeats of a consensus heptapeptide sequence (Zorio and Bentley, 2004; Sims et al., 2004). The CTD consensus sequence is highly conserved in eukaryotes, with the number of repeats varying from 26 in *S. cerevisiae* to 52 in humans (Bentley, 2002). CTD has a key role in the regulation of several steps of transcription and pre-mRNA processing, being required for transcriptional activation and repression, elongation, efficient capping, splicing, and cleavage/polyadenylation of RNA transcripts (Bentley, 2002; Proudfoot, 2004). This domain was specially designed for RNA Pol II interaction with transcription and processing machinery. The CTD of RNA Pol II undergoes extensive phosphorylation and dephosphorylation during transcription, and its functions are closely associated to its dynamic phosphorylation state, controlling the degree of RNA Pol II association to different co-factors during transcription steps (Lee and Young, 2000; Zorio and Bentley, 2004). Several kinases, and some phosphatases, are implicated in the phosphorylation state of CTD. Phosphorylated CTD has an important role in recruiting the mRNA capping enzyme to the nascent transcript, where it starts the mRNA capping soon after promoter clearance (Lee and Young, 2000; Dvir, 2002; Proudfoot, 2004). Furthermore, phosphorylated CTD interactions between yeast cleavage and poly(A) factors have been demonstrated biochemical and genetically (Proudfoot et al., 2002).

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When we examined the basal transcriptional machinery compounds of *P. brasiliensis* and compared them to the *S. cerevisiae* MIPS database (http://mips.gsf.de), we have found several related PbAESTs. However, many Pol II subunits, as well as other transcriptional factors, were absent from this transcriptome analysis, probably reflecting the growth phase from where the cDNA libraries used for ESTs generation were constructed, as well as the low abundance of some of these transcripts. The main findings from the *P. brasiliensis* transcriptome analyses are described below.

The analyses of *P. brasiliensis* transcriptome revealed two of the 12 *S. cerevisiae* RNA Pol II core subunits. The PbAEST 141 is ortholog to Rpb3, which encodes a 35-kDa polypeptide similar to the α subunit of prokaryotic RNA polymerase, while PbAEST 4986 is ortholog to Rpb7. In *S. cerevisiae*, Rpb7 along with the Rpb4 subunit, form a complex with roles in the stress response and in the initiation of transcription, and are accumulated during the stationary phase of cell growth (Lee and Young, 2000). In spite of the predicted association of those RNA Pol II subunits, we have not found any PbAEST related to Rpb4 in *P. brasiliensis*.

GENERAL TRANSCRIPTION FACTORS

Transcription of eukaryotic genes requires the assembly of RNA Pol II and GTFs on the promoter, to form a PIC (Martinez, 2002). The polymerase itself cannot recognize promoter sequences and initiate transcription without its association to GTFs. These proteins recognize several core promoter DNA elements, such as the TATA box, the initiator, and other regulatory elements. RNA Pol II holoenzyme is composed of all basal and regulatory transcription factors, as well as chromatin remodeling complexes, and DNA repair and replication factors (Buratowski, 2000). The basal class II transcription complex comprehends Pol II and six GTFs, including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (reviewed by Martinez, 2002). Among these GTFs, TFIID is the main sequence-specific DNA-binding component; the other GTFs enter the PIC primarily through protein-protein interactions. TFIID is composed of the TATA-box-binding protein (TBP) and multiple TBP-associated factors (TAFIIs) (Green, 2000; Albright and Tjian, 2000). Comparisons made with the *S. cerevisiae* genome have revealed orthologs to TBP, and at least one subunit of each TFII in *P. brasiliensis* transcriptome, the only exception is TFIIB, as summarized in Table 1.

ELONGATION

We have found four PbAESTs involved in transcript elongation in the *P. brasiliensis* transcriptome, including two subunits of the well-studied elongation factor, TFIIS. The TFIIS protein stimulates RNA Pol II to recover from an arrested state and resume elongation by inducing the polymerase to cleave its nascent transcript, repositioning the new RNA's 3' end within the polymerase catalytic center (Arndt and Kane, 2003). The extensive phosphorylation of CTD is known to be the transcriptional switch from initiation to elongation (Proudfoot et al., 2002; Arndt and Kane, 2003). One of the several kinases and phosphatases implied in CTD phosphorylation state and RNA Pol II activities is CTD kinase 1. We have found two orthologs of CTD kinase 1 (CTD-K1) subunits in *P. brasiliensis* transcriptome. This enzyme plays an important role in transcriptional elongation, creating a more processive form of RNA polymerase by CTD phosporylation (Jona et al., 2001; Kobor and Greenblatt, 2002).

Table 1. RNA polymerase II (Pol II)-related *Paracoccidioides brasiliensis* assembled expressed sequence tags (PbAESTs).

Transcription components	Ortholog name	PbAESTs in *Paracoccidioides brasiliensis*
Initiation		
RNA Pol II	Rpb3, Rpb7	141, 4986
TFIIA	Toa1	3276
TFIIH	Ssl1, Ssl2, Tfb4	1201, 1455, 5531, 4076
TFIIE	Tfa1	3184
TFIIF	Taf14; Tfg1, Tfg2	4539, 3608, 5615
TFIID	Taf5, Taf6, Taf10, Taf11, Taf13, Taf14, Spt15	2097, 1051, 2295, 5105, 2193, 4539, 1232
Core SRB/mediator	Gal11, Srb7, med6, med7, med8, Taf14, Nut2	614, 1366, 3859, 5667, 278, 4539, 2217
Swi/Snf complex	Swi3, Arp9, Snf12	5033, 3852, 2075
CCAAT-binding factor complex	Hap3, Hap5, Hapx, pirin	1426, 2423, 3390, 1662
SAGA complex	Gcn5, Ngg1, spt3, Taf5, Taf6, Taf10, Tra1	5286, 2230, 2150, 2097, 1051, 2295, 3460
CCR4-NOT	Ccr4, Caf4, Caf16, Not5	3262, 1416, 841, 4136
Elongation		
TFIIK complex	Ctk1, Ctk2	4774
Elongator complex	Elp2	5580
Elongation factors	Dst1, Spt4, Spt5	203, 1503, 1271
Transcription factors		
General transcription factors	Nif3, Tup1, Spt10, Cmbf, Hta2, Sef1, Cdc73, Cpr6, Egd2, Sub1, Ssn3, Rvb1, Yap3, Ask10	4950, 4855, 4744, 4427, 2103, 4232, 3297, 277, 620, 770, 4403, 5166, 4548, 1104
Metabolism regulation	MetR, Gal83, Met28, Upc2, Cha4, Nmra, Spb1, CPCA, Arg2, Ppr1, Met30, Gzf3, Pho85, Pho80, Gal1, Dal81	1140, 122, 1558, 1714, 215, 2166, 2239, 5296, 5449, 4942, 4588, 2859, 3718, 2646, 619
Pheromone response/mating	Mcm1, Mat1-1, NsdD	1259, 2475, 5614
Telomeric silencing	Hst4, Sas10, Dot1, Asf1	152, 1595, 3396, 851
Stress response	Skn7, Ask10, Yap1, Bcy1	2533, 1104, 1731, 947
Cell-cycle control	Swd1, Mbp1	2517, 968
Effectors of cell-signaling pathways	Crz1, Tpk2, PkaR	2408, 3488, 947
Circadian clock	WC2, ccg8	5269, 3877
Virulence	Bcy1, Yap1, Yap3, Cutinase transcription factor1, Mdr1, Tao3, rox1	947, 1731, 4548, 4516, 3937, 4745, 4188
Poorly characterized		1295, 1822, 1199, 5677, 1508, 5009, 271, 704, 3390, 3911, 4523

CHROMATIN REMODELING COMPLEX

Chromatin-modifying enzymes are components of multiple complexes involved in transcription initiation and elongation, making a link between chromatin structure and transcription regulation. The proteins that are involved are part of three major complexes: SWI/SNF, mediator and SAGA (reviewed by Kadonaga, 1998; Oki et al., 2004). SWI/SNF and the histone acetylation complex SAGA share several activities during transcriptional activation in *S. cerevisiae*. Many of the promoters that require one complex require the other as well. The SWI/SNF complex acts through noncovalent modifications of chromatin, and has an apparent mass of about 2 Mda, being composed of approximately 11 subunits. We have found three PbAESTs in *P. brasiliensis* transcriptome related to the SWI/SNF complex (Table1). The SAGA histone acetyltransferase/transcriptional adaptor complex is composed of multiple transcriptional regulators, including Ada, Spt, and TAFII proteins (Kadonaga, 1998; Ehrenhofer-Murray, 2004). We found seven PbAESTs similar to SAGA components, including an ortholog of GCN5 acetylase-histone acetyltransferase that normally acts by repressing transcription, playing an important role in chromatin structure or assembly (Kadonaga, 1998; Lee and Young, 2000).

We have also found four *S. cerevisiae* orthologs related to elements of the evolutionarily conserved CCR4-NOT complex, CAF4, CAF16, CCR4 and NOT5 in *P. brasiliensis* transcriptome. This complex was shown to be involved in several aspects of mRNA formation, including repression and activation of transcription initiation, control of mRNA elongation, deadenylation reaction and subsequent mRNA degradation (Denis and Chen, 2003).

Orthologs to components of other complexes involved in eukaryotic transcription were also found in *P. brasiliensis* transcriptome. These include subunits of other remodeling chromatin machineries, components of transcriptional silencing complex, and orthologs of transcription factors related to cell cycle and effector cell signaling pathways and metabolic control, as shown in Table 1.

mRNA transcription is usually the most regulated step in the pathway between a gene and its functional encoded protein. DNA-binding proteins, called transcription factors, mediate this regulation, in eukaryotes. Several PbAESTs, which encode transcription factors, have been found in *P. brasiliensis* transcriptome and will be highlighted here, since they are known to regulate processes as important as pheromone response and mating, stress response, circadian clock and virulence in other fungi.

PARACOCCIDIOIDES BRASILIENSIS SEXUAL CYCLE

We found three PbAESTs that encode transcription factors possibly related to sexual development. The first one is a homolog of MCM1, an MADS box protein, which interacts with co-factors regulating several cellular processes including mating in *S. cerevisiae* (Mead et al., 2002). The other factors are homologues to the mating-type protein MAT-1 and to NsdD, from *Aspergillus nidulans*. The NsdD gene of *A. nidulans* encodes a putative GATA-type transcription factor that is accumulated during the sexual development of this fungus. Deletion of NsdD resulted in no fruiting bodies formation, even under conditions that preferentially promoted sexual development; while over-expression lead to the formation of sexual-specific cells, even in conditions that normally block sexual development, suggesting an important role in the

activation of sexual development in *A. nidulans* (Han et al., 2001). *P. brasiliensis* is classified as an ascomycete, even though no sexual cycle has ever been observed. These three PbAESTs and a putative MAT-2 encoding sequence are evidence of either a yet to be observed sexual cycle, or of an ancestor which reproduced sexually.

The analysis of *C. albicans* genome data revealed the presence of mating-type genes in this organism, which was later confirmed experimentally, when a rare kind of mating between two strains of this fungus was described (Hull et al., 2000; Magee and Magee, 2000). Dyer and colleagues (2003) have also described mating-type genes, MAT-1 and MAT-2, in asexual aspergilli, showing a latent potential for sexuality in this group, although most *Aspergillus* species are only known to reproduce asexually. In addition to the genes described above, the finding of other transcription factors regulated by signal transduction pathways (Fernandes et al., in this issue, pages 216-231), strongly suggest, a not yet experimentally observed sexual cycle of *P. brasiliensis*.

VIRULENCE-RELATED TRANSCRIPTION FACTORS

Among transcriptional factors associated to putative virulence gene expression, we have found seven PbAESTs, with two of special interest: YAP1, the cutinase transcription factor 1-α, and ROX1. YAP1 is a transcriptional activator involved in drug resistance and oxidative stress response in *Saccharomyces cerevisiae* (Fernandes et al., 1997; Jamieson, 1998). Multicopies of this gene were found in transformants resistant to the iron chelators 1,10-phenanthroline and 1-nitroso-2-naphtol, as well as to a variety of drugs, including 4-nitroquinoline-*N*-oxide, *N*-methyl-*N*-nitro-*N*-nitrosoguanine, triaziquone, sulfomethuron methyl, and cyclohex-imide (Schnell et al., 1992; Stephen et al., 1995; Rodrigues-Pousada et al., 2004). If this gene plays a similar role in *P. brasiliensis,* it could be involved in fungal oxidative stress survival inside the phagolysosomal environment of macrophages.

Cutinase, an extracellular fungal enzyme, is known to be essential for the penetration of the cuticle by phytopathogens (Li et al., 2002). The presence of cutinase transcription factor in *P. brasiliensis* could either be interpreted as an adaptation to the fungus' natural habitat or a similar role in *P. brasiliensis* pathogenesis. It has been proposed by Steenbergen and Casadevall (2003), first in *Cryptococcus neoformans* and later in other pathogenic fungi, that many virulence factors affecting host-pathogen interactions were selected during evolution because they improve in some way the fitness of such organisms in their natural environment. This hypothesis has been reinforced by preliminary studies showing that interactions of some pathogenic fungi with soil amoebae resemble interactions of those organisms with macrophages (Steenbergen and Casadevall, 2003; Steenbergen et al., 2004). Having in mind that *P. brasiliensis* is a soil fungus and that it does not depend on animal infection for reproduction, the same mechanisms described for the origin and maintenance of virulence attributes in other pathogenic fungi should be also expected for this organism.

The regulator of filamentous growth and virulence, ROX1, is related to the regulation of hypoxic genes under anaerobic conditions in *S. cerevisiae*. Its closest relative in *C. albicans*, named Rfg1, does not appear to play a role in the regulation of the hypoxia response but, instead, it controls filamentous growth and virulence (Kadosh and Johnson, 2001; Liu, 2001, 2002). If this gene really has similar functions in *P. brasiliensis*, it would be a very interesting target for further characterization.

PRE-mRNA MODIFICATIONS AND SPLICING

The transcription of protein-encoding genes produces pre-mRNAs that undergo a number of co- and post-transcriptional modifications prior to the transport of functional mRNAs to the cytoplasm. These processing reactions include capping, polyadenylation and splicing.

The capping reaction occurs after the synthesis of 20-40 nucleotides (Coppola et al., 1983), and is performed by three enzymes acting in succession (Moteki and Price, 2002). In *S. cerevisiae*, the enzyme RNA 5' triphosphatase (*cet1*) hydrolyzes the triphosphate of the 5' nucleotide to a diphosphate, then a guanylyltransferase (GT, *ceg1*) catalyzes the fusion of a GMP moiety, generated from GTP, to the first nucleotide of the pre-mRNA via an unusual 5'-5' triphosphate linkage. In *S. cerevisiae*, GT is found associated to Cet1, which interacts with the CTD of RNA Pol II, probably as a heterotrimer (Shuman, 2001). The methyltransferase (*abd1*), methylates the N7 position of the GMP, acting as a positive effector, modulating the RNA Pol II function (Shroeder et al., 2004). In the *P. brasiliensis* transcriptome, the PbAESTs 1564 and 2314 were annotated as orthologs to the *S. cerevisiae abd1* (methyltransferase) and *ceg1* (GT) genes, respectively (Table 2). In the nucleus, the 5' cap structure is recognized by the cap binding complex (CBC). The 5' CBC seems to play several important roles, such as stabilization of the mRNA (since it represents an obstacle for 5'-3' exonucleases), exportation of the mature mRNA to the cytoplasm and participation in the first steps of the translation initiation process (Beelman and Paker, 1995, reviewed by Wilkie et al., 2003). As shown in Table 2, the PbAESTs 1652 and 1173 were found as homologues of *S. cerevisiae Cbc2* and *Sto1* genes, which encode the small and large subunits of CBC, respectively.

Table 2. mRNA processing-related *Paracoccidioides brasiliensis* assembled expressed sequence tags (PbAESTs).

Function	PbAEST
Capping	
Transferase	2314
Methyltransferase	1564
Ligation and transport	1652, 1173
Decapping	114, 4793, 776, 2188, 1126, 439, 4814, 988
Polyadenylation	
Cleavage	1221, 1334, 1221
Synthesis	5359, 4230, 3921, 1314
Enhancer	5058, 1050

Different from what is seen in prokaryotes, the eukaryotic mRNA 3' end is not generated in the final steps of transcription, since a termination signal does not exist. Instead, it is formed by co- and post-transcriptional modifications, mediated by an apparatus that is coupled to the transcription machinery (Humphrey and Proudfoot, 1988; Bentley, 2002; Proudfoot, 2004). With the exception of very few genes, like the replication-dependent histone genes, the 3'-end formation of almost all protein-encoding mRNAs is carried out by the reactions of cleavage and poly(A) addition (Sheets and Wickens, 1989). The poly(A) tail addition is directed by several cis-acting sequence elements in the pre-mRNA and by several trans-acting protein factors

(Wickens, 1990; Proudfoot, 1991). In contrast to higher eukaryotes, where the polyadenylation-positioning element is the highly conserved hexanucleotide AAUAAA, *S. cerevisiae* uses degenerate and complex signals to direct these reactions. The major sequence elements described in *S. cerevisiae* comprehend blocks of sequences, such as: efficiency element, positioning element, the nucleotides at the poly(A) addition site, and the upstream and downstream U-rich elements (Zhao et al., 1999; Dichtl and Keller, 2001). The positioning element resembles the mammalian sequence in at least two aspects: AAUAAA is one of the several related sequences that function as positioning elements, promoting the pre-mRNA cleavage approximately 20 nucleotides downstream, preferentially at Py(A)n sequences (Wahle and Rüegsegger, 1999). In yeast, the cleavage and polyadenylation-specifying factor makes essential RNA-protein contacts with signals encompassing the cleavage site (Dichtl and Keller, 2001). The affinity of cleavage and polyadenylation-specifying factor for the AAUAAA hexamer is further enhanced by the binding of the cleavage stimulatory protein complex (CstF), to a GU-rich motif, called downstream sequence element (Zhao et al., 1999). Two additional proteins, cleavage factors I (CFI) and II, recognize the processing signals of pre-mRNA and perform the endonucleolytic cleavage, whereas CFI, polyadenylation factor I (PFI), and the single-polypeptide poly(A) polymerase (PAP, pap1p) are required for poly(A) tail synthesis (Chen and Moore, 1992). In *S. cerevisiae*, the CFI complex contains five subunits, Rna14, Rna15, Paf11, Clp1, and Hrp1, all necessary and sufficient for cleavage reactions when mixed with CFII, which is composed by the subunits Cft1, Cft2, Brr5, and Pta1. As shown in Table 2, the PbAESTs 1334 and 1221 are orthologs to *S. cerevisiae* genes encoding for Hrp1 and Pcf11 CFI subunits, respectively. At this moment, no PbAESTs related to CFII subunits were annotated in *P. brasiliensis* transcriptome. The synthesis of the poly(A) tail is catalyzed by PAP in two stages: first, a sequence of nearly 10 adenine nucleotides is added to the mRNA 3' end; a very fast reaction that depends entirely on the hexamer AAUAAA; then, the poly(A) tail is elongated up to a length of 200 nucleotides in higher eukaryotes, and 70-90 in fungi (Jacobson and Peltz, 1996; Wahle and Rüegsegger, 1999). During the elongation step, the poly(A) binding protein binds stoichiometrically to every 20 A residues, playing important roles in mRNA biogenesis and function (reviewed by Wilkie et al., 2003). The interaction of poly(A) binding protein and Fip1, a subunit of PFI, with PAP seems to be important in PAP specificity and processivity, probably controlling the poly(A) tail size (Zhelkovsky et al., 1998). Several PbAESTs related to components of PFI and PFII complexes have been found in the *P. brasiliensis* transcriptome. Three PbAESTs were described as related to PFI; PbAESTs 5359 and 4230 keep similarity with the *S. cerevisiae Pfs2* gene and PbAEST 3921 with *Yhs1*, while PbAEST 1314 was annotated as an ortholog to *S. cerevisiae Yth1* gene, encoding for a PFII component (Table 2).

Interrupted protein-encoding genes are often found in eukaryotes. In *S. cerevisiae,* only 5% of the genes are interrupted, frequently by one intron (Käufer and Potashkin, 2000), while in higher eukaryotes most genes contain several intervening sequences. Pre-mRNA splicing is an essential and highly precise process, responsible for the removal of introns and joining of exons in the primary transcript, producing a mature mRNA (Padgett et al., 1986). However, different combinations of removal or retention of specific introns/exons, can occur during the splicing of many pre-mRNAs, creating a diverse array of mature mRNAs from a single pre-mRNA, a process called alternative splicing (Maniatis, 1991; Green, 1991; Roberts and Smith, 2002). Alternative splicing is a highly regulated event that generates related proteins, or protein isoforms, that commonly differ within specific functional domains. Nowadays, this process has

emerged as a mechanism, at least in part, responsible for the increased complexity observed at mRNA and protein levels. Noteworthy is the finding that the human genome seems to have only around 40,000 protein-encoding genes, which is much less than previously estimated and only two or three times more than the number of genes in *Caenorhabditis elegans* and *Drosophila melanogaster* (Venter et al., 2001). In this sense, alternative splicing could significantly contribute to the vertebrate biological complexity when compared with simpler organisms.

In general, introns present three short cis-acting sequences that are important in splicing reactions: GU at the 5'-end (the 5' splice site or splice donor site), AG at the 3' end (the 3' splice site or splice acceptor site), as defined by the GT-AG rule, and the sequence called branch site, frequently located 20-50 bases upstream of the 3' splice site (Reed and Maniatis, 1985; Green, 1986). The pre-mRNA splicing is carried out by two transesterification reactions, where a lariat intermediate is formed by a 2'-5' phosphodiester linkage between the 5' splice site and an invariant adenosine in the branch site. After the next cleavage at the 3' splice site, the lariat, representing the intron to be removed, will be degraded and the two exons joined. The reactions involved in pre-mRNA splicing are performed by the spliceosome, a highly organized apparatus composed of five small nuclear RNAs (snRNAs) and nearly hundred proteins (Steitz et al., 1988; Ruby and Abelson, 1991; Green, 1991). The spliceosome assembles in a precise stepwise model onto each individual intron by performing extensive RNA-RNA, RNA-protein and protein-protein interactions in the steps of recognition and removal of the intron, with the subsequent ligation of adjacent exons (Hastings and Krainer, 2001).

After the completion of the *S. cerevisiae* genome project, all genes related to the 105 splicosomal proteins, besides the five snRNAs, have been defined and subdivided in several functional categories, as described in the MIPS databank (http://mips.gsf.de). In the *P. brasiliensis* transcriptome, 65 PbAESTs orthologs to *S. cerevisiae* basal splicing machinery genes were found, as described in Table 3. The PbAEST 1420, annotated as an ortholog to *S. cerevisiae snp1* gene, which encodes a U1 snRNP-specific protein, was shown to be a *P. brasiliensis* mycelial phase differentially expressed gene by electronic subtraction and microarray experiments (data not shown). Although in *S. cerevisiae* Snp1p is physically associated with proteins that regulate splicing (Awasthi et al., 2001), at the moment we cannot predict the role of this mycelial-phase gene of *P. brasiliensis*.

According to Table 3, except for U4-U6 specific proteins, we have found representatives of all other categories of *S. cerevisiae* splicing machinery, corroborating the idea that we have an almost complete overview of the expressed genes in *P. brasiliensis*. However, it is important to notice that probably many of the splicing regulated factors were underscored, since some of them could be *P. brasiliensis* specific.

In addition to the events required to generate mature mRNA to be transported to the cytoplasm, where it can be translated, its degradation is also of central importance for the control of gene expression. In this sense, mRNA degradation is an essential step in mRNA metabolism, with several pathways accomplishing this task in the cell. One of the major mechanisms of mRNA degradation in eukaryotes involves the deadenylation-dependent decapping. This mechanism involves a gradual shortening of the poly(A) tail, mediated by a 3' to 5' exonucleolytic reaction, followed by the 5' cap removal (decapping), resulting in a fast mRNA degradation reaction from both directions (Beelman and Parker, 1995; Jacobson and Peltz, 1996; Schwartz and Parker, 1999). A family of proteins, referred as Lsm proteins (like Sm), has been identified as affecting the deccaping step of this degradatation pathway (Thaurun et al.,

Table 3. Splicing machinery categories and their corresponding *Paracoccidioides brasiliensis* assembled expressed sequence tags (PbAESTs).

Categorization	Total PbAESTs	PbAEST numbers
Core snRNP proteins	14	36, 439, 603, 776, 988, 1126, 1220, 1607, 1641, 2161, 2188, 2642, 4793, 4814
U1 snRNP specific proteins	5	448, 1420, 2457, 3466, 3927
U2 snRNP specific proteins	7	842, 1525, 1775, 1787, 2351, 2452, 4693
U5 snRNP specific proteins	1	3708
Proteins associated with the *prp19* complex	3	731, 3800, 4806
Proteins with demonstrated roles in splicing	5	1129, 3043, 3424, 4761, 5467
Other splicing regulated proteins	30	41, 207, 326, 479, 488, 735, 1173, 1243, 1314, 1339,1657, 1760, 1912, 2023, 2341, 2490, 2671, 2836, 3317, 3612, 3964, 4185, 4578, 4703, 5066, 5100, 5152, 5194, 5270, 5373

2004). These proteins contain the "Sm motif", also found in proteins that assemble the snRNPs involved in pre-mRNA splicing, suggesting that Lsm proteins are important modulators of RNA metabolism in both the cytoplasm and the nucleus (revised by He and Parker, 2000; Thaurun et al., 2004). Seven PbAEST homologues to *S. cerevisiae* genes encoding Lsm proteins were found in *P. brasiliensis* transcriptome, as shown in Table 3. Additionally, the PbAEST 114, encoding a homologue of *S. cerevisiae* Edc1, that functions as an enhancer of the decapping reaction, was also found.

POST-TRANSCRIPTIONAL GENE SILENCING

An important mechanism of gene expression regulation, recently described in eukaryotic cells, is the post-transcriptional gene silencing (PTGS), or RNA silencing, which is a sequence-specific mRNA degradation mechanism (Baulcombe, 2002). A conserved mechanism recognizes double-stranded RNA as a sequence-specific signal to induce the silencing of cognate genes (Sharp, 2001). This phenomenon has been reported in plants as co-suppression, in fungi as "quelling", and in nematodes as RNA interference, and it has become clear that these mechanisms are closely related (Nishikura, 2001). The analysis of the presence of *P. brasiliensis* PTGS machinery was made by comparison of the genes described for *Neurospora crassa* quelling pathway (Galagan et al., 2003) with *P. brasiliensis* transcriptome.

A stepwise model is described in several organisms for the RNA silencing pathway. In the first step, double-stranded RNAs are processed by DICER, an RNase III-like enzyme, into 21-25 nucleotide RNA fragments known as small interfering RNAs (siRNAs) (Hamilton and Baulcombe, 1999). These siRNAs will be part of a multicomponent nuclease complex (RISC), functioning as a guide, by specific base-pairing interactions, for the positioning of RISC in the mRNA substrate (Hammond et al., 2000). This ribonucleoprotein complex (RISC), of around 500 kDa, is composed of several subunits, including the argonaute family members (Hammond

et al., 2001). In *P. brasiliensis* transcriptome analysis, the PbAESTs 5236 and 5450 were found as orthologs to *N. crassa* genes *sms-2* and *qde-2*, respectively (Table 4). Furthermore, a probable candidate of RNase III-like enzyme (dcl-2) was also identified in *P. brasiliensis* transcriptome as PbAEST 4507 (Table 4).

Table 4. *Paracoccidioides brasiliensis* assembled expressed sequence tags (PbAESTs) possibly involved in post-transcriptional gene silencing in *P. brasiliensis*.

Predicted function	Pathway	*N. crassa* genes	PbAESTs
RNA-directed RNA polymerase	Quelling	*qde-1*	-
	Meiotic silencing	*sad-1*	-
	Unknown	*rrp-3*	4284
Argonaute-like	Quelling	*qde-2*	5450
	Meiotic silencing	*sms-2*	5236
RNAse III-like	Quelling	*dcl-2*	4507
	Meiotic silencing	*sms-3*	-

The data on *Neurospora crassa* post-transcriptional gene silencing were as described by Galagan et al. (2003).

Additionally, the amplification of the target RNA, by a cellular RNA-directed RNA polymerase, using siRNAs as primers, is proposed to increase the effectiveness of the RNA silencing mechanism. A protein sequence homologue to RNA-directed RNA polymerase has been reported to be required for efficient silencing in fungal, nematode and plant systems (Sijen et al., 2001). We also found an RNA-directed RNA polymerase that could be related to PTGS in this fungus (Table 4).

Since the blockage of a gene is a widespread methodology employed to understand its function, we believe that the description of genes possibly related to the silencing machinery will open new perspectives in the establishment of protocols for the study of gene function in this fungus.

RNA POLYMERASE I AND III TRANSCRIPTION MACHINERIES

Eukaryotic RNA Pol I and III transcribe a limited set of genes. However, the activities of Pol I and Pol III are responsible for more than 80% of total RNA synthesis in growing cells (Paule and White, 2000). RNA Pol I apparatus synthesizes the large rRNAs, while RNA Pol III transcribes genes encoding small RNA molecules, including the tRNAs, 5S rRNA, and other stable untranslated RNAs involved in RNA processing, such as U6 spliceosomal RNA and RNase P RNAs (Harismendy et al., 2003). Although the transcription factors are different, there are some similarities in the mechanism used by these two enzymes (Paule and White, 2000).

In all eukaryotes, RNA polymerases are complex enzymes constituted of 12 or more subunits (Paule and White, 2000), of which five subunits are shared among Pol I, II and III, and two other subunits are shared between Pol I and Pol III. The presence of common subunits may be related to the regulation of the three transcription machineries in a coordinated manner (Mann et al., 1987). Several Pol I and III subunits, including one that is common to both enzymes, were identified in *P. brasiliensis* transcriptome by BLAST (Table 5).

Table 5. *Paracoccidioides brasiliensis* open reading frames related to rRNA and tRNA synthesis.

Function	PbAESTs
DNA-directed RNA Pol I subunits	1868, 2558, 3507, 5349
DNA-directed RNA Pol III subunits	643, 2144, 2558, 3768
TFIIIC (transcription initiation factor) subunits	1740, 3654, 4766
TATA-binding protein	1232

PbAESTs = *Paracoccidioides brasiliensis* assembled expressed sequence tags; Pol = polymerase.

In addition to the Pol I and III holoenzymes, several transcription factors have been described as being important in the transcription mechanism. The essential transcription factors required for Pol III to initiate transcription of tRNA genes are TFIIIC and TFIIIB. These factors have been best characterized in *S. cerevisiae* (Lodish et al., 2000). *S. cerevisiae* TFIIIC is composed of six essential subunits: TFC1p, TFC3p, TFC4p, TFC6p, TFC7p, and TFC8p (Huang and Maraia, 2001). Three of these TFIIIC subunits were identified in *P. brasiliensis* transcriptome by BLAST (Table 5), including a protein ortholog of TFC4p, a large protein that may provide a flexible link connecting TFIIIC and TFIIIB in the initiation complex. The primary function of TFIIIC is to recruit TFIIIB, a process that has been well-characterized in yeast (Paule and White, 2000). *S. cerevisiae* TFIIIB is a complex of three polypeptides, one of which is a TBP. A candidate for the *S. cerevisiae* TBP ortholog was identified in *P. brasiliensis* transcriptome, as shown in Table 5. Once TFIIIB has interacted with a specific gene promoter through the TBP subunit, Pol III can bind and initiate the transcription (Lodish et al., 2000).

Ribosome biogenesis and the control of rDNA transcription through RNA Pol I are known to be essential for cell growth. The fact that the level of protein synthesis is proportional to ribosome abundance in cells, and that the biogenesis of ribosomes is in part dependent upon the production of rRNA, makes the regulation of ribosome synthesis an important process for cell growth and proliferation (Reeder, 1999). However, no Pol I transcription factors were detected in the *P. brasiliensis* transcriptome analysis. This could be explained by the fact that the cDNA library was made using cells in the stationary phase of growth. In this phase, their reproductive ability is gradually lost, suggesting as a consequence, that the Pol I regulation apparatus was absent at that moment. In addition, Pol I transcription in *S. cerevisiae* is known to be regulated by nutrient starvation (Kief and Warner, 1981; James and Zomerdijk, 2004). Since the *in vitro* condition of growth implies in less nutrients available, and rRNA synthesis appears to be regulated accordingly, we suggest that this was the reason why we could not found the transcripts (PbAESTs) related to Pol I regulation apparatus.

RNA POL I TRANSCRIPT MATURATION

In the nucleolus, RNA Pol I generates a large rRNA polycistronic precursor (pre-rRNA) that contains the sequences for the mature ribosomal RNAs (18S, 5.8S, 25-28S rRNA), two external transcribed spacers and two internal transcribed spacers. The remaining part of the rDNA repeat is formed by two nontranscribed spacers, separated by the 5S rRNA gene. This is independently transcribed in the opposite direction as a precursor by RNA Pol III.

In all organisms, rRNA synthesis is not achieved by simple transcription of the individual rRNA species, requiring a complex series of post-transcriptional processing steps. rRNA transcription and most maturation steps in eukaryotes take place, for the most part, in a specialized nuclear sub-compartment called nucleolus, which is also the site of ribosome synthesis (Kressler et al., 1999). rRNA is synthesized by the Pol I apparatus in the fibrillar centers and then processed and assembled into ribosomes in the surrounding granular regions (Shaw and Jordan, 1995).

Maturation of the pre-rRNA is accompanied by the addition of a large number of covalent modifications, mainly by pseudouridylation of uridine residues and 2'-O-ribose methylation of sugar moiety (Kiss, 2001; Bachellerie et al., 2002; Filipowicz and Pogacic, 2002; Decatur and Fournier, 2003). Further maturation of the pre-rRNA involves removal of the transcribed spacers in a series of processing steps carried out by endo- and exoribonucleases. Pre-rRNA processing has been most extensively studied in the yeast *S. cerevisiae* (Venema and Tollervey, 1999). In this organism, the earliest detectable 35S pre-rRNA is subjected to chemical modification and subsequently cleavage steps, which in an early stage removes 5' external transcribed spacer and separates the 18S precursor (20S) from the 25S and 5.8S precursor (27S). The 20S pre-rRNA is subsequently transported to the cytoplasm, where it is cleaved, yielding the mature 18S rRNA, while the 27S pre-rRNA undergoes several processing steps to yield the mature 5.8S and 25S rRNAs (Udem and Warner, 1973; Venema and Tollervey, 1999). Since it appears unlikely that ribosome synthesis in *S. cerevisiae* yeast will be substantially more complex than in other eukaryotes, we believe that the principles discovered in this organism could be applied to *P. brasiliensis* and to other eukaryotes. We found several orthologs to *S. cerevisiae* rRNA processing related genes in the transcriptome of *P. brasiliensis*, including 3'-5' exoribonuclease, ATP-dependent RNA helicase, rRNA (adenine-N6,N6-)-dimethyltransferase, rRNA pseudouridine synthase, mitochondrial rRNA methyltransferase, multifunctional nuclease, fibrillarin, and others, as shown in Table 6.

The two types of chemical modification steps in the pre-rRNA are guided by two large families of small nucleolar RNAs (snoRNA), that function by correctly positioning the modification enzymes on the pre-rRNA by pairing with the target site, while the catalytic function is provided by a common protein enzyme, methylase or pseudouridinesynthase, associated with the snoRNA (Bachellerie et al., 2002). The two snoRNA guide families have been identified in a wide spectrum of eukaryal species, ranging from metazoans to yeasts, plants, and kinetoplastid protozoan. They are called: the box C/D and box H/ACA snoRNAs. Most members of the two snoRNA families guide the 2'-O-ribose methylations and pseudouridylations of rRNA (Balakin et al., 1996; Venema and Tollervey, 1999). Box C/D snoRNAs are associated with the common box C/D *S. cerevisiae* proteins Nop1p (fibrillarin in human), Nop56p, Nop58p, and Snu13p. Nop1p/fibrillarin is the enzyme that catalyzes the methylation reaction. In *S. cerevisiae* cells with a Nop1p-specific mutation, the methylation process is blocked, resulting in a clearly inhibited 60S ribosome synthesis (Venema and Tollervey, 1999). Nop1p and Nop58p orthologs are also represented in the *P. brasiliensis* transcriptome. Also, box H/ACA snoRNAs are associated with *S. cerevisiae* core proteins Nhp2p, Nop10p, Gar1p, and Cbf5p, of which the latter is thought to catalyze the isomerization of the uridine residue (Venema and Tollervey, 1999). Gar1p and Cbf5p orthologs were found in the *P. brasiliensis* transcriptome.

In *S. cerevisiae*, U3 and U14 are the only box C/D snoRNAs required for the cleavage reactions (Li et al., 1990; Hughes and Ares, 1991; Venema and Tollervey, 1999), and both

Table 6. *Paracoccidioides brasiliensis* open reading frames related to rRNA processing and modification.

Function	Description	PbAEST	*Saccharomyces cerevisiae* correspondent protein
Processing of the 35S pre-rRNA	5'-3' exoribonuclease	94	Rat1p
Common snoRNP proteins - Box C/D	involved in 2'-*O*-methylation of the pre-rRNA	1013, 4199	Nop1p, Nop58p
Common snoRNP proteins - Box H/ACA	involved in pseudouridylations of the pre-rRNA	451 and 2382, 848	Cbf5p, Gar1p
Base methylation	rRNA adenine dimethyltransferase	4480	Dim1p
	rRNA guanosine methyltransferase	2745	Pet56p
Required for 18S rRNA synthesis	U3 snoRNP protein Specific U3 snoRNP protein	4711, 1546, 627 1899 and 3997, 84, 5752, 635	Utp4p, Utp5p, Utp11p Imp3p, Imp4, Sof1p, Mpp10p
Involved in 18S formation	ATP-dependent RNA helicase	537, 2014, 1132, 41	Rok1p, Rrp3p, Dbp2p, Mak5p
	Nuclear localization signal	1362	Nsr1p
Cytoplasmic maturation of 20S pre-rRNA	5'-3' exoribonuclease	94	Rat1p
Synthesis of the 5.8S and 25S rRNAs	5'-3' exoribonuclease	328, 526	Rrp4p, Dis3p
Biogenesis of 60S ribosome subunit	Required for efficient 60S ribosome subunit biogenesis	3238	Nip7p

PbAEST = *Paracoccidioides brasiliensis* assembled expressed sequence tag.

function through direct base pairing with the pre-rRNA. The U3 snoRNP contains at least five proteins that do not appear to be components of any other snoRNP - Imp3p, Imp4, Sof1p, Mpp10p, and Rrp9p (Hughes and Ares, 1991; Dunbar et al., 1997; Lee and Baserga, 1999; Venema et al., 2000). We found three PbAESTs related to U3 snoRNP protein and all of its specific snoRNP orthologs, except Rrp9p (Table 6). U14 snoRNPs were not captured in our transcriptome analysis. Electronic subtraction and cDNA microarray analyses using mycelium and yeast *P. brasiliensis* ESTs (data not shown) revealed one component of U3 snoRNP, which is differentially expressed in the yeast phase specific, and is ortholog to *S. cerevisiae mpp10* gene. Mpp10p is a truncated version of the U3 snoRNP protein and it specifically inhibits cleavage at sites A1 and A2, without affecting processing at A0 (Lee and Baserga, 1997).

The number of modified sites increases with the complexity of the organism, although modification patterns show evolutionary conservation, and most sites modified in the yeast *S. cerevisiae* are also modified in vertebrates. The role of these modifications is not exactly clear; however, they can promote base-pairing interactions and influence ribosome activities (Maden and Hughes, 1997; Decatur and Fournier, 2002, 2003; King et al., 2003).

RNA POL III TRANSCRIPT MATURATION

The products of RNA Pol III should be processed and modified after transcription. All of the primary transcription products of tRNA genes studied so far are larger than the final products (Lodish et al., 2000). This occurs because tRNA genes are also interrupted by introns. The tRNA splicing reaction in yeast occurs in three steps, each catalyzed by distinct enzymes. In the first step, the pre-tRNA is cleaved by an endonuclease, which is a heterotetramer whose subunits have molecular masses of 54 (*SEN54*), 44 (*SEN2*), 34 (*SEN34*), and 15 kDa (*SEN15*). Each of these genes has proved to be essential for cell viability (Abelson et al., 1998). Two of these subunits were found in *P. brasiliensis* transcriptome (Table 7). The products of the endonuclease reaction are substrates for the next step, catalyzed by the tRNA ligase. An ortholog of this enzyme was identified in *P. brasiliensis* transcriptome, as shown in Table 7. The last processing step is catalyzed by the phosphotransferase (Abelson et al., 1998).

Table 7. *Paracoccidioides brasiliensis* open reading frames related to tRNA processing and modification.

Function	PbAEST
tRNA splicing endonuclease (subunits)	221, 2568
tRNA ligase	4998
RNA binding protein (chaperone)	1187
related to RNase P and RNase MRP	818, 1611
tRNA-specific adenosine deaminase 1	20
tRNA-pseudouridine synthase	2382, 5219
N2,N2-dimethylguanine tRNA methyltransferase	3038
tRNA isopentenyltransferase	403
C1-tetrahydrofolate synthase	116
Other related activities	1238, 2273, 5082

PbAEST = *Paracoccidioides brasiliensis* assembled expressed sequence tag.

Additionally, the precursor tRNA transcripts need to be cleaved by ribonuclease P (RNase P) to mature its 5' ends. RNase P is essential for cell viability, and this protein shares four subunits with RNase MRP, the related rRNA processing enzyme. The degree of structural similarity between nuclear RNase P and RNase MRP suggests that pre-tRNA and pre-rRNA processing pathways are partially overlapped (Stolc and Altman, 1997; Chamberlain et al., 1998). We found two proteins that are related to RNase P and RNase MRP complexes in the *P. brasiliensis* transcriptome (Table 7).

Other important proteins necessary for normal processing of many RNA Pol III transcripts were found in *P. brasiliensis* transcriptome (Table 7). One example is the *S. cerevisiae* protein ortholog Lhp1p, related to a chaperone, that binds nascent RNA molecules, including

pre-tRNAs, pre-5SrRNA and pre-U6 RNA (Rinke and Steitz, 1982). In *P. brasiliensis*, like *S. cerevisiae*, this protein may function as a molecular chaperone, facilitating the correct fate of newly synthesized RNA Pol III transcripts.

Many chemical modifications in the tRNA precursor are also required, such as the addition of methyl and isopentenyl groups, and the conversion of uridines into pseudouridines (Lodish et al., 2000). Open reading frames for several enzymes related to tRNA modification have been found in *P. brasiliensis* transcriptome, such as pseudouridine synthases, tRNA isopentenyltransferase, N2,N2-dimethylguanine tRNA methyltransferase, tRNA-specific adenosine deaminase 1, and C1-tetrahydrofolate synthase (Table 7).

CONCLUDING REMARKS

The transcriptome of *P. brasiliensis*, a dimorphic fungus responsible for a severe systemic mycosis in Latin America, has provided important information about this microorganism's physiology. Several aspects related to the biology of this fungus can be inferred by comparative analyses of PbAESTs, with sequences deposited in different databases. The RNA biogenesis apparatus is very similar to those of other eukaryotes, such as *S. cerevisiae*. The results presented in this report could be used as a starting point for further studies in order to better understand the molecular biology of this important human pathogen.

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REFERENCES

Abelson, J., Trotta, C.R. and **Li, H.** (1998). tRNA splicing. *Biol. Chem. 21*: 12685-12688.
Albright, S.R. and **Tjian, R.** (2000). TAFs revisited: more data reveal new twists and confirm old ideas. *Gene 242*: 1-13.
Arndt, K.M. and **Kane, C.M.** (2003). Running with RNA polymerase: eukaryotic transcript elongation. *Trends Genet. 19*: 543-550.
Awasthi, S., Palmer, R., Castro, M., Mobarak, C.D. and **Ruby, S.W.** (2001). New roles for the Snp1 and Exo84 proteins in yeast pre-mRNA splicing. *J. Biol. Chem. 33*: 31004-31015.
Bachellerie, J.P., Cavaille, J. and **Huttenhofer, A.** (2002). The expanding snoRNA world. *Biochimie 84*: 775-790.
Balakin, A.G., Smith, L. and **Fournier, M.J.** (1996). The RNA world of the nucleolus: two major families of small RNAs defined by different box elements with related functions. *Cell 86*: 823-834.
Baulcombe, D. (2002). RNA silencing. *Curr. Biol. 12*: R82-R84.
Beelman, C.A. and **Paker, R.** (1995). Degradation of mRNA in eukaryotes. *Cell 81*: 179-183.
Bentley, D. (2002). The mRNA assembly line: transcription and processing machines in the same factory. *Curr. Opin. Cell Biol. 14*: 336-342.
Buratowski, S. (2000). Snapshots of RNA polymerase II transcription initiation. *Curr. Opin. Cell Biol. 12*: 320-325.
Chamberlain, J.R., Lee, Y., Lane, W.S. and **Engelke, D.R.** (1998). Purification and characterization of the nuclear RNase P holoenzyme complex reveals extensive subunit overlap with RNase MRP. *Genes Dev. 11*: 1678-1690.
Chen, J. and **Moore, C.** (1992). Separation of factors required for cleavage and polyadenylation of yeast pre-mRNA. *Mol. Cell Biol. 12*: 3470-3481.
Coppola, J.A., Field, A.S. and **Luse, D.S.** (1983). Promoter proximal pausing by RNA polymerase II *in vitro*: transcripts shorter than 20 nucleotides are not capped. *Proc. Natl. Acad. Sci. USA 80*: 1251-1255.
Decatur, W.A. and **Fournier, M.J.** (2002). rRNA modifications and ribosome function. *Trends Biochem.*

Sci. 27: 344-351.

Decatur, W.A. and **Fournier, M.J.** (2003). RNA-guided nucleotide modification of ribosomal and other RNAs. *J. Biol. Chem. 278*: 695-698.

Denis, C.L. and **Chen, J.** (2003). The CCR4-NOT complex plays diverse roles in mRNA metabolism. *Prog. Nucleic Acid. Res. Mol. Biol. 73*: 221-250.

Dichtl, B. and **Keller, W.** (2001). Recognition of polyadenylation sites in yeast pre-mRNA by cleavage and polyadenylation factor. *EMBO J. 12*: 3197-3209.

Dunbar, D.A., Wormsley, S., Agentis, T.M. and **Baserga, S.J.** (1997). Mpp10p, a U3 small nucleolar ribonucleoprotein component required for pre-18S rRNA processing in yeast. *Mol. Cell. Biol. 17*: 5803-5812.

Dvir, A. (2002). Promoter escape by RNA polymerase II. *Biochim. Biophys. Acta 1577*: 208-223.

Dyer, P.S., Paoletti, M. and **Archer, D.B.** (2003). Genomics reveals sexual secrets of *Aspergillus. Microbiology 149*: 2301-2303.

Ehrenhofer-Murray, A.E. (2004). Chromatin dynamics at DNA replication, transcription and repair. *Eur. J. Biochem. 271*: 2335-2349.

Felipe, M.S., Andrade, R.V., Petrofeza, S.S., Maranhão, A.Q., Torres, F.A., Albuquerque, P., Arraes, F.B., Arruda, M., Azevedo, M.O., Baptista, A.J., Bataus, L.A., Borges, C.L., Campos, E.G., Cruz, M.R., Daher, B.S., Dantas, A., Ferreira, M.A., Ghil, G.V., Jesuino, R.S., Kyaw, C.M., Leitão, L., Martins, C.R., Moraes, L.M., Neves, E.O., Nicola, A.M., Alves, E.S., Parente, J.A., Pereira, M., Poças-Fonseca, M.J., Resende, R., Ribeiro, B.M., Saldanha, R.R., Santos, S.C., Silva-Pereira, I., Silva, M.A., Silveira, E., Simões, I.C., Soares, R.B., Souza, D.P., De-Souza, M.T., Andrade, E.V., Xavier, M.A., Veiga, H.P., Venâncio, E.J., Carvalho, M.J., Oliveira, A.G., Inoue, M.K., Almeida, N.F., Walter, M.E., Soares, C.M. and **Brigido, M.M.** (2003). Transcriptome characterization of the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis* by EST analysis. *Yeast 20*: 263-271.

Felipe, M.S., Andrade, R.V., Arraes, F.B., Nicola, A.M., Maranhao, A.Q., Torres, F.A., Silva-Pereira, I., Pocas-Fonseca, M.J., Campos, E.G., Moraes, L.M., Andrade, P.A., Tavares, A.H., Silva, S.S., Kyaw, C.M., Souza, D.P., Network P., Pereira, M., Jesuino, R.S., Andrade, E.V., Parente, J.A., Oliveira, G.S., Barbosa, M.S., Martins, N.F., Fachin, A.L., Cardoso, R.S., Passos, G.A., Almeida, N.F., Walter, M.E., Soares, C.M., Carvalho, M.J. and **Brigido, M.M.** (2005). Transcriptional profiles of the human pathogenic fungus *Paracoccidioides brasiliensis* in mycelium and yeast cells. *J. Biol. Chem. 280*: 24706-24714.

Fernandes, L., Rodrigues-Pousada, C. and **Struhl, K.** (1997). Yap, a novel family of eight bZIP proteins in *Saccharomyces cerevisiae* with distinct biological functions. *Mol. Cell Biol. 17*: 6982-6993.

Filipowicz, W. and **Pogacic, V.** (2002). Biogenesis of small nucleolar ribonucleoproteins. *Curr. Opin. Cell Biol. 14*: 319-327.

Franco, M. (1987). Host-parasite relationships in paracoccidioidomycosis. *J. Med. Vet. Mycol. 25*: 5-18.

Galagan, J.E., Calvo, S.E., Borkovich, K.A., Selker, E.U., Read, N.D., Jaffe, D., FitzHugh, W., Ma, L.J., Smirnov, S., Purcell, S., Rehman, B., Elkins, T., Engels, R., Wang, S., Nielsen, C.B., Butler, J., Endrizzi, M., Qui, D., Ianakiev, P., Bell-Pedersen, D., Nelson, M.A., Werner-Washburne, M., Selitrennikoff, C.P., Kinsey, J.A., Braun, E.L., Zelter, A., Schulte, U., Kothe, G.O., Jedd, G., Mewes, W., Staben, C., Marcotte, E., Greenberg, D., Roy, A., Foley, K., Naylor, J., Stange-Thomann, N., Barrett, R., Gnerre, S., Kamal, M., Kamvysselis, M., Mauceli, E., Bielke, C., Rudd, S., Frishman, D., Krystofova, S., Rasmussen, C., Metzenberg, R.L., Perkins, D.D., Kroken, S., Cogoni, C., Macino, G., Catcheside, D., Li, W., Pratt, R.J., Osmani, S.A., DeSouza, C.P.C., Glass, L., Orbach, M.J., Berglund, J.A., Voelker, R., Yarden, O., Plamann, M., Seiler, S., Dunlap, J., Radford, A., Aramayo, R., Natvig, D.O., Alex, L.A., Mannhaupt, G., Ebbole, D.J., Freitag, M., Paulsen, I., Sachs, M.S., Lander, E.S., Nusbaum, C. and **Birren, B.** (2003). The genome sequence of the filamentous fungus *Neurospora crassa. Nature 422*: 859-868.

Gow, N.A.R., Brown, A.J.P. and **Odds, F.C.** (2002). Fungal morphogenesis and host invasion. *Curr. Opin. Microbiol. 5*: 366-371.

Green, M. (1986). Pre-mRNA splicing. *Ann. Rev. Genet. 20*: 671-708.

Green, M.R. (1991). Biochemical mechanisms of constitutive and regulated pre-mRNA splicing. *Ann. Rev. Cell Biol. 7*: 559-599.

Green, M.R. (2000). TBP-associated factors (TAFIIs): multiple, selective transcriptional mediators in common complexes. *Trends Biochem. Sci. 25*: 59-63.

Hahn, S. (2004). Structure and mechanism of the RNA polymerase II transcription machinery. *Nat. Struct. Mol. Biol. 11*: 394-403.

Hamilton, A.J. and **Baulcombe, D.C.** (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science 286*: 950-952.

Hammond, S.M., Bernstein, E., Beach, D. and **Hannon, G.J.** (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature 404*: 293-296.

Hammond, S.M., Boettcher, S., Caudy, A.A., Kobayashi, R. and **Hannon, G.J.** (2001). Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science 293*: 1146-1150.

Han, K.H., Han, K.Y., Yu, J.H., Chae, K.S., Jahng, K.Y. and **Han, D.M.** (2001). The nsdD gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. *Mol. Microbiol. 41*: 299-309.

Harismendy, O., Gendrel, C.G., Soularue P., Gidrol, X., Sentenac, A., Werner, M. and **Lefebvre, O.** (2003). Genome-wide location of yeast RNA polymerase III transcription machinery. *EMBO J. 22*: 4738-4747.

Hastings, M.L. and **Krainer, A.R.** (2001). Pre-mRNA splicing in the new millennium. *Curr. Opin. Cell Biol. 13*: 302-309.

He, W. and **Parker, R.** (2000). Functions of Lsm proteins in mRNA degradation and splicing. *Curr. Opin. Cell Biol. 12*: 346-350.

Howe, K.J. (2002). RNA polymerase II conducts a symphony of pre-mRNA processing activities. *Biochim. Biophys. Acta 1577*: 308-324.

Huang, Y. and **Maraia, R.J.** (2001). Comparison of the RNA polymerase III transcription machinery in *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and human. *Nucleic Acids Res. 29*: 2675-2690.

Hughes, J.M.X. and **Ares, M.J.** (1991). Depletion of U3 small nucleolar RNA inhibits cleavage in the 5'external transcribed spacer of yeast pre-ribosomal RNA and impairs formation of 18S ribosomal RNA. *EMBO J. 10*: 4231-4239.

Hull, C.M., Raisner, R.M. and **Johnson, A.D.** (2000). Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. *Science 289*: 307-310.

Humphrey, T. and **Proudfoot, N.J.** (1988). A beginning to the biochemistry of polyadenylation. *Trends Genet. 4*: 243-245

Jacobson, A. and **Peltz, S.W.** (1996). Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. *Annu. Rev. Biochem. 65*: 693-739.

James, M.J. and **Zomerdijk, J.C.** (2004). Phosphatidylinositol 3-kinase and mTOR signaling pathways regulate RNA polymerase I transcription in response to IGF-1 and nutrients. *J. Biol. Chem. 279*: 8911-8918.

Jamieson, D.J. (1998). Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast 114*: 1511-1527.

Jona, G., Wittschieben, B.O., Svejstrup, J.Q. and **Gileadi, O.** (2001). Involvement of yeast carboxy-terminal domain kinase I (CTDK-I) in transcription elongation *in vivo*. *Gene 267*: 31-36.

Kadonaga, J.T. (1998). Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines. *Cell 92*: 307-133.

Kadosh, D. and **Johnson, A.D.** (2001). Rfg1, a protein related to the *Saccharomyces cerevisiae* hypoxic regulator Rox1, controls filamentous growth and virulence in *Candida albicans*. *Mol. Cell. Biol. 21*: 2496-2505.

Käufer, N.F. and **Potashkin, J.** (2000). Analysis of the splicing machinery in fission yeast - a comparison with budding yeast and mammals. *Nucleic Acid Res. 16*: 3003-3010.

Kief, D.R. and **Warner, J.R.** (1981). Coordinate control of syntheses of ribosomal ribonucleic acid and ribosomal proteins during nutritional shift-up in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 1*: 1007-1015.

King, T.H., Liu, B., McCully, R.R. and **Fournier, M.J.** (2003). Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. *Mol. Cell. 11*: 425-435.

Kiss, T. (2001). Small nucleolar RNA-guided post-transcriptional modifications of cellular RNAs. *EMBO J. 20*: 3617-3622.

Kobor, M.S. and **Greenblatt, J.** (2002). Regulation of transcription elongation by phosphorylation. *Biochim. Biophys. Acta 1577*: 261-275.

Kressler, D., Linder, P. and **de La Cruz, J.** (1999). Protein trans-acting factors involved in ribosome biogenesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 19*: 7897-7912.

Lee, S.J. and **Baserga, S.J.** (1997). Functional separation of pre-rRNA processing steps reveled by truncation of the U3 small nucleolar ribonucleoprotein component, Mpp10. *Proc. Natl. Acad. Sci. USA 94*: 13536-13541.

Lee, S.J. and **Baserga, S.J.** (1999). Imp3p and Imp4p: two specific components of the U3 small nucleolar ribonucleoprotein that are essential for pre 18S rRNA processing. *Mol. Cell. Biol. 19*: 5441-5452.

Lee, T.I. and **Young, R.A.** (2000). Transcription of eukaryotic protein-coding genes. *Annu. Rev. Genet. 34*:

77-137.

Li, D., Sirakova, T., Rogers, L., Ettinger, W.F. and **Kolattukudy, P.E.** (2002). Regulation of constitutively expressed and induced cutinase genes by different zinc finger transcription factors in *Fusarium solani* f. sp. pisi (*Nectria haematococca*). *J. Biol. Chem. 277*: 7905-7912.

Li, H.V., Zagorski, J. and **Fournier, M.J.** (1990). Depletion of U14 small nucleolar RNA (snR128) disrupts production of 18S rRNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 10*: 1145-1152.

Liu, H. (2001). Transcriptional control of dimorphism in *Candida albicans*. *Curr. Opin. Microbiol. 4*: 728-735.

Liu, H. (2002). Co-regulation of pathogenesis with dimorphism and phenotypic switching in *Candida albicans*, a commensal and a pathogen. *Int. J. Med. Microbiol. 292*: 299-311.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and **Darnell, J.** (2000). *Molecular Cell Biology*. 4th edn. New York, 10010.

Maden, B.E. and **Hughes, J.M.** (1997). Eukaryotic ribosomal RNA: the recent excitement in the nucleotide modification problem. *Chromosoma 105*: 391-400.

Magee, B.B. and **Magee, P.T.** (2000). Induction of mating in *Candida albicans* by construction of MTLa and MTLalpha strains. *Science 289*: 310-313.

Maniatis, T. (1991). Mechanisms of alternative pre-mRNA splicing. *Science 251*: 33-34.

Mann, C., Buhler, J.M., Treich, I. and **Sentenac, A.** (1987). RPC40, a unique gene for a subunit shared between yeast RNA polymerases A and C. *Cell 48*: 627-637.

Martinez, E. (2002). Multi-protein complexes in eukaryotic gene transcription. *Plant Mol. Biol. 50*: 925-947.

Mead, J., Bruning, A.R., Gill, M.K., Steiner, A.M., Acton, T.B. and **Vershon, A.K.** (2002). Interactions of the Mcm1 MADS box protein with cofactors that regulate mating in yeast. *Mol. Cell. Biol. 22*: 4607-4621.

Mitsuzawa, H. and **Ishihama, A.** (2004). RNA polymerase II transcription apparatus in *Schizosaccharomyces pombe*. *Curr. Genet. 44*: 287-294.

Moteki, S. and **Price, D.** (2002). Functional coupling of capping and transcription of mRNA. *Mol. Cell. 10*: 599-609.

Nishikura, K. (2001). A short primer on RNAi: RNA-directed RNA polymerase acts as a key catalyst. *Cell 107*: 415-418.

Oki, M., Valenzuela, L., Chiba, T., Ito, T. and **Kamakaka, R.T.** (2004). Barrier proteins remodel and modify chromatin to restrict silenced domains. *Mol. Cell. Biol. 24*: 1956-1967.

Padgett, R.A., Grabowski, P.J., Konarska, M.M., Seiler, S. and **Sharp, P.A.** (1986). Splicing of messenger RNA precursors. *Ann. Rev. Biochem. 55*: 1119-1150.

Paule, M.R. and **White, R.J.** (2000). Transcription by RNA polymerases I and III. *Nucleic Acids Res. 28*: 1283-1298.

Proudfoot, N.J. (1991). Poly(A) signals. *Cell 64*: 671-674.

Proudfoot, N.J. (2004). New perspectives on connecting messenger RNA 3' end formation to transcription. *Curr. Opin. Cell Biol. 16*: 272-278.

Proudfoot, N.J., Furger, A. and **Dye, M.J.** (2002). Integrating mRNA processing with transcription. *Cell 108*: 501-512.

Reed, R. and **Maniatis, T.** (1985). Intron sequences involved in lariat formation during pre-mRNA splicing. *Cell 41*: 95-105.

Reeder, R.H. (1999). Regulation of RNA polymerase I transcription in yeast and vertebrates. *Prog. Nucleic Acid Res. Mol. Biol. 62*: 293-327.

Restrepo, A., McEwen, J.G. and **Castaneda, E.** (2001). The habitat of *Paracoccidioides brasiliensis*: how far from solving the riddle? *Med. Mycol. 39*: 233-241.

Rinke, J. and **Steitz, J.A.** (1982). Precursor molecules of both human 5S rRNA and tRNAs are bound by a cellular protein reactive with anti-La lupus antibodies. *Cell 29*: 149-159.

Roberts, G.C. and **Smith, C.W.J.** (2002). Alternative splicing: combinatorial output from the genome. *Curr. Opin. Chem. Biol. 6*: 375-383.

Rodrigues-Pousada, C.A., Nevitt, T., Menezes, R., Azevedo, D., Pereira, J. and **Amaral, C.** (2004). Yeast activator proteins and stress response: an overview. *FEBS Lett. 567*: 80-85.

Ruby, S.W. and **Abelson, J.** (1991). Pre-messenger RNA splicing in yeast. *Trends Genet. 7*: 79-85.

Sakurai, H., Mitsuzawa, H., Kimura, M. and **Ishihama, A.** (1999). The Rpb4 subunit of fission yeast *Schizosaccharomyces pombe* RNA polymerase II is essential for cell viability and similar in structure to the corresponding subunits of higher eukaryotes. *Mol. Cell. Biol. 19*: 7511-7518.

Schnell, N., Krems, B. and **Entian, K.D.** (1992). The PAR1 (YAP1/SNQ3) gene of *Saccharomyces cerevisiae*, a c-jun homologue, is involved in oxygen metabolism. *Curr. Genet. 21*: 269-273.

Schroeder, S., Zorio, D., Schwer, B., Shuman, S. and **Bently, D.** (2004). A function of yeast mRNA cap methyltransferase, Abd1, in transcription by RNA polymerase II. *Mol. Cell. 13*: 377-387.

Schwartz, D. and **Paker, R.** (1999). Mutations in translation initiation factors lead to increased rates of deadenylation of mRNA in *Saccharomyces cerevisiae. Mol. Cell. Biol. 19*: 5247-5256.

Sharp, P. (2001). RNA interference 2001. *Genes Dev. 15*: 485-490.

Shaw, P.J. and **Jordan, E.G.** (1995). The nucleolus. *Annu. Rev. Cell. Dev. Biol. 11*: 93-121.

Sheets, M.D. and **Wickens, M.** (1989). Two phases in the addition of a poly(A) tail. *Genes Dev. 3*: 1401-1412.

Shuman, S. (2001). Structure, mechanism, and evolution of the mRNA capping apparatus. *Prog. Nucleic Acid Res. Mol. Biol. 66*: 1-40.

Sijen, T., Fleenor, J., Simmer, F., Thijssen, K.L., Parrish, S., Timmons, L., Plasterk, R.H.A. and **Fire, A.** (2001). On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell 107*: 465-476.

Sims, R.J., **Mandal, S.S.** and **Reinberg, D.** (2004). Recent highlights of RNA-polymerase-II-mediated transcription. *Curr. Opin. Cell. Biol. 16*: 263-271.

Steenbergen, J.N. and **Casadevall, A.** (2003) The origin and maintenance of virulence for the human pathogenic fungus *Cryptococcus neoformans. Microbes Infect. 5*: 667-675.

Steenbergen, J.N., Nosanchuk, J.D., Malliaris, S.D. and **Casadevall, A.** (2004). Interaction of *Blastomyces dermatitidis*, *Sporothrix schenckii*, and *Histoplasma capsulatum* with *Acanthamoeba castellanii. Infect. Immun. 72*: 3478-3488.

Steitz, J.A., Black, D.L., Gerke, V., Parker, K.A., Krämer, A., Frendewey, D. and **Keller, W.** (1988). Functions of the abundant U-snRNPs. In: *Structure and Functions of Major and Minor Small Nuclear Ribonucleoprotein Particles* (Birnstiel, M.L., ed.). Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, pp. 115-154.

Stephen, D.W., Rivers, S.L. and **Jamieson, D.J.** (1995). The role of the YAP1 and YAP2 genes in the regulation of the adaptive oxidative stress responses of *Saccharomyces cerevisiae. Mol. Microbiol. 16*: 415-423.

Stolc, V. and **Altman, S.** (1997). Rpp1, an essential protein subunit of nuclear RNase P required for processing of precursor tRNA and 35S precursor rRNA in *Saccharomyces cerevisiae. Genes Dev. 18*: 2414-2425.

Thaurun, S., He, W., Mayes, A.E., Lennertz, P., Begg, J.D. and **Parker, R.** (2004). Yeast Sm-like proteins function in mRNA decapping and decay. *Nature 404*: 515-518.

Udem, S.A. and **Warner, J.R.** (1973). The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. *J. Biol. Chem. 248*: 1412-1416.

Venema, J. and **Tollervey, D.** (1999). Ribosome synthesis in *Saccharomyces cerevisiae. Annu. Rev. Genet. 33*: 261-311.

Venema, J., Faber, A.W., Vos, H.R., Pluk, H., van Venrooij, W.J. and **Raué, H.A.** (2000). Yeast Rrp9p is an evolutionarily conserved U3 snoRNP protein essential for early pre-rRNA processing cleavages and requires box C for its association. *RNA 6*: 1660-1671.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., Gocayne, J.D., Amanatides, P., Ballew, R.M., Huson, D.H., Wortman, J.R., Zhang, Q., Kodira, C.D., Zheng, X.H., Chen, L., Skupski, M., Subramanian, G., Thomas, P.D., Zhang, J., Gabor Miklos, G.L., Nelson, C., Broder, S., Clark, A.G., Nadeau, J., McKusick, V.A., Zinder, N., Levine, A.J., Roberts, R.J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A.E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T.J., Higgins, M.E., Ji, R.R., Ke, Z., Ketchum, K.A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G.V., Milshina, N., Moore, H.M., Naik, A.K., Narayan, V.A., Neelam, B., Nusskern, D., Rusch, D.B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M.L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferriera, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y.H., Romblad, D., Ruhfel, B.,

Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N.N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J.F., Guigo, R., Campbell, M.J., Sjolander, K.V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y.H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wen, M., Wu, D., Wu, M., Xia, A., Zandieh, A. and **Zhu, X.** (2001). The sequence of the human genome. *Science 5507*: 1304-1351.

Wahle, E. and **Rüegsegger, U.** (1999). 3'-End processing of pre-mRNA in eukaryotes. *FEMS Microbiol. Rev. 23*: 277-295.

Wickens, M. (1990). How the messenger got its tail: addition of poly(A) in the nucleus. *Trends Biochem. Sci. 15*: 277-281.

Wilkie, G.S., Dickson, K.S. and **Gray, N.K.** (2003). Regulation of mRNA translation by 5'- and 3'-UTR-binding factors. *Trends Biochem. Sci. 28*: 182-188.

Zhao, J., Hyman, L. and **Moore, C.** (1999). Formation of mRNA 3' ends in eukaryotes: mechanism, regulation and interrelationships with other steps in mRNA synthesis. *Microbiol. Mol. Biol. Rev. 63*: 405-445.

Zhelkovsky, A., Helmling, S. and **Moore, C.** (1998). Precessivity of the *Saccharomyces cerevisiae* poly(A) polymerase requires interactions at the carboxyl-terminal RNA binding domain. *Mol. Cell. Biol. 18*: 5942-5951.

Zorio, D.A. and **Bentley, D.L.** (2004). The link between mRNA processing and transcription: communication works both ways. *Exp. Cell Res. 296*: 91-97.