The role of DSC1 components cdc10\(^+\), rep1\(^+\) and rep2\(^+\) in MCB

gene transcription at the mitotic G1-S boundary in fission yeast

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Abstract In this paper, we describe the transcription profile of a group of genes at the G1-S boundary of fission yeast in synchronously dividing mitotic cells, under a variety of different conditions. This transcription profile is unaffected in cells where either cdc10\(^-\) or cdc10-C4 are constitutively overexpressed. In contrast, overexpression of either rep1\(^+\) or rep2\(^+\) results in constitutive expression of MCB-regulated genes, suggesting that these polypeptides have important regulatory properties in controlling MCB transcription. Finally, we examine the pattern of MCB-regulated transcription in cells where the G1 period is extended. Surprisingly, we find that the wee1-50 mutation causes MCB transcription throughout the cell cycle, whereas cells re-fed after nitrogen starvation have normal expression patterns. The implications of these observations for understanding MCB-regulated transcription are discussed.

Keywords Fission yeast · cdc10 · rep1 · rep2

Introduction

The cell cycle is the process which cells undergo when they duplicate and divide. Many different forms of control have been elucidated that regulate this process, such as intracellular location, protein kinase activity, specific proteolytic degradation and transcription. In a number of cases, transcription has been found to play an important role in controlling cell cycle progress. An example of this is the regulation of expression of the G1 cyclins, CLN1 and CLN2, in the budding yeast Saccharomyces cerevisiae; elevated transcription of CLN1 or CLN2 results in the acceleration of the cell cycle, while reduced transcription slows the cycle down (Johnston and Lowndes 1992).

Cell cycle-regulated transcription has been well studied in the fission yeast, Schizosaccharomyces pombe. A group of genes, including cdc22\(^+\), cig2\(^+\), cdt1\(^+\) and cdc18\(^+\), are transiently expressed at the beginning of S-phase and their products are required, either directly or indirectly, for DNA synthesis; these genes encode products such as ribonucleotide reductase and a G1 cyclin (Kelly et al. 1993; Fernandez-Sarabia et al. 1993; Connolly and Beach 1994; Hofmann and Beach 1994).

The molecular components that co-ordinate cell cycle transcription in fission yeast have been discovered; they comprise a transcription complex binding to common, repeated DNA sequences in the promoters of the four S-phase genes. The transcription factor has been named DSC1 (DNA synthesis control) and contains the products of the cdc10\(^+\), res1\(^+\), res2\(^+\), rep1\(^+\) and rep2\(^+\) genes (Lowndes et al. 1992; Caligiuri and Beach 1993; Miyamoto et al. 1994; Sugiyama et al. 1994; Zhu et al. 1994; Nakashima et al. 1995).

DSC1 binds to so-called MCB sequences (MluI cell cycle box); coincidentally, the sequence ACGCGT is the same as the recognition sequence of the restriction enzyme, MluI). Such sequences are present in the promoters of cdc22\(^+\), cdc18\(^+\), cdt1\(^+\) and cig2\(^+\), all of which are maximally expressed at the G1-S boundary during the mitotic cell cycle. Furthermore, it has been demonstrated that isolated MCBs confer cell cycle-regulated transcription to a heterologous gene in a reporter plasmid, strongly implicating MCBs in regulating G1-S transcription in fission yeast (Lowndes et al. 1992).

DSC1 and MCBs control the expression of a group of genes required for entry into S-phase. This implies that control of activity of this transcription apparatus is itself important for cell cycle progress. At present, there is little
understanding of how DSC1 and MCBs work together to control cell cycle-regulated transcription in fission yeast. It is believed that Cdc10p plays a central role in this regulation, partly because it is absolutely required for cell cycle progression (Nurse and Bissett 1981), but also because various cdc10 mutations have profound effects on MCB-dependent gene expression (Fernandez-Sarabia et al. 1993; Kelly et al. 1993; Hofmann and Beach 1994; McInerney et al. 1995; Baum et al. 1997). These mutations suggest that Cdc10p may have both positive and negative roles in controlling cell cycle-regulated genes. Other recent experiments have suggested that the partners to Cdc10p in DSC1, Res1p and Res2p, also have roles in cell cycle-regulated transcription, with Res1p activating and Res2p repressing transcription (Baum et al. 1997; Whitehall et al. 1999).

We sought to investigate the role of DSC1 in controlling MCB-regulated gene transcription by studying the effect of overexpressing various components of the complex on cell cycle transcription. Furthermore, we examined the effect of extending the G1 phase on periodic expression of genes at the G1-S boundary. The implications of these results for mechanisms of G1-S transition in fission yeast are discussed.

**Materials and methods**

**Media and general techniques**

General molecular procedures were performed as described by Sambrook et al. (1989), while the media used for the propagation of *S. pombe* were as described by Moreno et al. (1991). The standard genetics procedures of Guzzi et al. (1974) and Kohli et al. (1977) were followed. The following strains were used in this study: 972 h− (GG 1), h− cdc25-22 leu1-32 ura4-D18 (GG 193), h− cdc2-33 leu1-32 (GG 194) and h− wee1-50 (GG 67). For all physiological experiments, cells were grown in minimal medium (EMM; Moreno et al. 1991), with shaking, at 25 °C or 32 °C. Temperature-sensitive mutants were incubated at the restrictive temperature of 36 °C in order to display their mutant phenotype.

Synchronisation of cells by reciprocal temperature shifts in the cdc25-22 mutants was achieved by growing cells in EMM to mid-exponential growth at 25 °C, before shifting to 36 °C for 4 h. Cells were then shifted back to 25 °C to enter the mitotic cell cycle in synchrony. Samples were subsequently removed at regular time intervals both for RNA extraction, and to measure septation indices by microscopic examination.

Populations of synchronously dividing fission yeast cells were prepared by use of a Beckman elutriator rotor (Creanor and Mitchison 1979). Cell numbers per millilitre of liquid culture were determined from samples fixed in a 0.1% formaldehyde/0.1% sodium chloride solution. Following sonication, cells were counted electronically with a Coulter counter. Flow cytometry analysis, using a fluorescence-activated cell sorter (FACS) was performed as previously described (McInerney et al. 1995), using the FACSscan system and the Lysis II analysis program (Becton Dickinson); and 10,000 cells were analysed at each time point. For nitrogen-starvation experiments, cells were grown to the mid-exponential phase of growth at 30 °C, washed three times in nitrogen-free EMM, and grown in this same medium for 15 h. Cells were then re-fed with nitrogen by the addition of 5 g NH4Cl 1−.

To overexpress genes using the pREP1 vector (Maundrell 1993) during a synchronous cell cycle, cells were grown in EMM with 5 μg thiamine μl−1 (mnt1− promoter “off”) to the mid-exponential stage of growth. Cells were washed three times in thiamine-free EMM and then grown for 16 h in EMM without thiamine (mnt1− promoter “on”), at the same temperature. Next day, cells were synchronised by reciprocal temperature shifts, as described above.

**DNA constructs**

The plasmids pREP1:rep1− and pREP1:rep2+ were made by amplifying the open reading frames of rep1− and rep2+ from cDNA by PCR, using oligonucleotides which gave an NdeI restriction site at the ATG and a BamHI site 3′ to the stop codon for each gene. This allowed the genes to be inserted in frame with the mnt1− promoter in the pREP1 series of plasmids (Maundrell 1993). The oligonucleotides used were as follows:

Rep1−: (1) 5′-CGTCATATGGAATTCCGAA-3′, 3′-CGTCATATGGAATTCCGAA-5′ and (2) 5′-GCCGAGGATCCCTAACGAC-3′ and (2) 5′-GCCGAGGATCCCTAACGAC-5′.

Rep2+: (1) 5′-CGTCATATGGAATTCCGAA-3′, 3′-CGTCATATGGAATTCCGAA-5′ and (2) 5′-GCCGAGGATCCCTAACGAC-3′ and (2) 5′-GCCGAGGATCCCTAACGAC-5′.

All amplified DNAs were confirmed by sequencing. The pREP1: cdc10− and pREP1: cdc10-C4 constructs have been described previously (McInerney et al. 1995).

**DNA and RNA manipulations**

*S. pombe* total RNA was prepared as described by McInerney et al. (1995) and Northern blot analysis was carried out using a GeneScreen membrane (NEN, Life Science Products, Boston, Mass.), following the manufacturer’s suggested protocol. DNA probes were labelled with [α-32P]dCTP, using the random hexanucleotide labelling procedure of Feinberg and Vogelstein (1983). Northern blots were hybridised with the following DNA probes made by PCR, using the corresponding oligonucleotides:

cdc22−: (1) 5′-GATCAATGATGCTAATGGAAT3′ and (2) 5′-GATCATCTGTAACAGAG3′, adh1−: (1) 5′-AAGACTTATGCACTTCGACCA3′ and (2) 5′-TTACTTGGGAAAGTGA3′, cdc18+: (1) 5′-GGTTGTCTACAATCCTGGAAG3′ and (2) 5′-CAGACACTGAAATGGA3′, AATGACATCC3′, cdc1−: (1) 5′-CCCCCGATTGAAAAAGTTGTA3′ and (2) 5′-CATGCGGAATGTTGTTTCC3′, cig2−: (1) 5′-CTCTATTTTCTAATGGCA3′ and (2) 5′-GAGATCAATGATGCTAATGGAAT3′, cdc10−: (1) 5′-GGACAGGCGCCGCTTCCACGTGGGACCA3′ and (2) 5′-ATAGACTTTCCTGGCAGCA3′, CATCTGTAACAGAG3′ and (2) 5′-TTACAATCTGCTAATGGCA3′ and (2) 5′-TTATAATCTCCTGTAA3′ and (2) 5′-CATTTTGTGAAATGGA3′ and (2) 5′-TAAAAGCGACCAGCATCTACGT3′, histone 2A1: (1) 5′-GTCGAGGGAATAATCTGGA3′ and (2) 5′-CAGCTCCCTGACTAGGTTC3′.

**Results**

Overexpression of cdc10− and cdc10-C4 have no effect on MCB gene transcription throughout the cell cycle

Previous experiments have demonstrated that MCB genes are controlled by a transcription factor complex, named DSC1, which contains the gene products of the cdc10−, res1−, res2−, rep1− and rep2+ (Lowndes et al. 1992; Caligiuri and Beach 1993; Miyamoto et al. 1994; Sugiyama et al. 1994; Zhu et al. 1994; Nakashima et al. 1995). We sought to address the regulatory role of Cdc10p in DSC1 by analysing the effect of overexpressing cdc10− on MCB genes throughout the cell